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ACETYLCHOLINE LEVEL OF RAT CEREBRAL CORTEX UNDER CONDITIONS OF ANOXIA AND HYPOGLYCEMIA

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(Received for publication February 15, 1943)

INTRODUCTION

THE IMPORTANCE of oxygen and glucose in the synthesis of acetylcholine (ACh) by brain slices has been demonstrated by Quastel, Tennenbaum and Wheatley (15) and by Mann, Tennenbaum and Quastel (11, 12). The *in vivo* synthesis of ACh by the superior cervical ganglion of the cat has also been shown to depend on an adequate supply of oxygen and glucose (7). However, attempts to demonstrate a change in the ACh level of the brain as a result of anoxia or hypoglycemia have thus far failed (9, 3). The present paper is an account of experiments which demonstrate, with reasonable certainty, that low atmospheric pressure (probably acting through anoxia), and insulin hypoglycemia, cause a decrease in the level of ACh in the cerebral cortex of the rat.

METHODS

Rats of the Sprague-Dawley albino strain were used. These ranged in weight from 110 to 300 g. but the majority weighed between 200 and 250 g. Anoxia was produced by subjecting rats to low atmospheric pressure. The chamber was evacuated in steps of 50 or 100 mm. Hg with pauses of 10 minutes between successive lowerings. At a stage when respiratory movements had just ceased (between 100 and 200 mm. Hg) the pressure was quickly returned to normal, the rat decapitated, and the cortex removed for extraction. Hypoglycemia was produced by fasting rats 16 to 22 hours, always including one night, then injecting insulin intramuscularly. Some were allowed to survive 30 minutes after the injection, some were sacrificed in convulsions and others in coma. All experiments were terminated by decapitation.

Three methods of extracting the ACh were tried. The trichloracetic acid method of Chang and Gaddum (1) and a dialysis method after Lissák (8) were not found entirely satisfactory for reasons to be given later. A rapid, cold-Ringer method was developed which extracted the free ACh and this was used in the majority of the experiments. Three methods of assay were also tried: the leech muscle preparation; the isolated, eserinized, frog heart (Straub preparation); and the isolated heart of the mollusc Venus mercenaria.‡ The leech muscle preparation was discarded as too slow and insensitive. In one series of experiments (Summer series) all extracts were tested simultaneously on the frog heart and the Venus heart by different persons. In the Fall series assays were made on only the frog heart, and by a third person. Each extract was matched several times with known concentrations of ACh. The stock solution of acetylcholine chloride was made up in 5 per cent NaH_2PO_4, sealed in ampoules, heated to boiling for 5 minutes and stored in the refrigerator. Stock ACh prepared in this manner undergoes about 10 per cent loss of activity in one year. All values for tissue ACh obtained in this investigation are expressed in terms of an equivalent weight of the free base and not of the chloride. Further details of procedure will be given as the separate experiments are considered.

* With the technical assistance of Sibyl Beckett, Jane E. Hyde and Todd C. Smith.
† Aided by a grant from the Milton Fund of Harvard University.
‡ Details of the method of assaying tissue extracts for ACh, employing the Venus heart (based on findings of Prosser, 14), will be described in a separate publication.

Results

1. *ACh in normal cortex.* MacIntosh (10) in a thorough analysis for ACh in the central and peripheral nervous systems of the cat and dog prepared his animals by injecting chloralose or chloralose-urethane and eserine. Extracts of tissues were made with trichloracetic acid. In order to determine the most satisfactory methods to employ in the experimental studies a number of procedures were tried on normal rats to test the effects of anaesthesia, injection of eserine, and different methods of extraction. Nembutal anaesthesia, with and without eserine, was tried, and three extraction procedures were tested:

(a) *Dialysis.* Rats were injected subcutaneously with 0.1 grain of nembutal. After deep anaesthesia the cortex was removed, weighed, and ground with silica in unbuffered frog heart Ringer (6.5 g. NaCl, 0.14 g. KCl, 0.12 g. $CaCl_2$, H_2O to 1 l.) plus eserine (physostigmine) sulphate 1:10,000. The volume was adjusted to 1 cc. for each 100 mg. of tissue. This was dialysed in a cellophane sac against an equal volume of unbuffered Ringer for 2 hours at 15°C. The fluid outside the sac was diluted with bicarbonate Ringer and assayed on the frog heart. Assay of the cortex from six rats gave an average value of 1.17γ ACh/gram of wet tissue. A similar procedure was repeated on four rats except that they received 1 cc. of 1:5000 eserine sulphate subcutaneously. This produced characteristic salivation and muscular tremors. Upon assay an average value of 1.35γ ACh/gram of cortex was obtained.

By using the Venus heart, which is apparently unaffected by substances other than ACh in tissue suspensions, at the great dilutions employed, it was possible to assay for ACh in the contents of the sac as well as in the surrounding fluid. A higher value of ACh was always found inside the sac even after several hours of dialysis. This suggested that some synthesis might be taking place during dialysis, or that some ACh was bound to large molecules which did not dialyze out, hence this method was considered unsatisfactory for the type of experiments to be done later.

(b) *Trichloracetic acid extraction.* Cortical tissue from five rats was extracted with 10 per cent trichloracetic acid after the method of Chang and Gaddum (1). Assays were made on both the frog heart and the Venus heart. Those on the former were not always satisfactory, probably due to occasional incomplete removal of all traces of acid and ether which have a deleterious effect on the frog heart. The greater sensitivity of the Venus heart allowed greater dilution, and assays on this preparation gave an average value of 1.6γ ACh/gram of cortex. This may be considered a fair estimate of the total ACh in the rat cortex.

(c) *Cold-Ringer extraction.* It was believed that the free* ACh of the cortex might be more labile than the bound and might increase or decrease in amount when there was no measureable change in total ACh. Hence a

* In the present discussion the "free ACh" may be defined as that part of the total ACh which is readily extractible in water in the presence of adequate amounts of eserine or prostigmine, but without employing a protein denaturant.

quick method was evolved to extract the free fraction. The removed cortex was placed in 2 cc. of ice cold, unbuffered frog heart Ringer with eserine 1:10,000; weighed, ground with silica in a chilled mortar and the volume adjusted to 1 cc. for each 100 mg. of cortex. The suspension was mixed well and centrifuged at high speed for 5 or 10 minutes. It was found that the length of time that the tissue suspension was mixed and allowed to stand before centrifuging affected the yield, hence for a given series this period was kept as constant as possible. After centrifuging, the supernatant fluid was poured off and stored at near O°C. until just before assaying, when it was diluted with bicarbonate Ringer, or sea water, depending on which heart preparation was to be used. With this procedure extracts were ready for assay within 20 to 30 minutes from the beginning of removal of the cortex.

Three rats under nembutal anaesthesia yielded an average value of 0.33 γ free ACh per g. of cortex when assayed on the frog heart and 0.43 γ when assayed on the Venus heart. Lower values for a given extract were consistently obtained with the frog heart due to the presence of small amounts of excitatory substances. These had no apparent effect on the Venus heart.

Seven normal rats decapitated with no previous treatment, gave an average value of 0.25γ free ACh/gram of cortex when assayed on the frog heart and 0.35γ when assayed on the Venus heart. Comparing this value obtained on the Venus heart with that of the total ACh by the trichloracetic acid method (1.6γ) it is seen that the free ACh constitutes about one fourth the total ACh of the rat cortex. Mann, Tennenbaum and Quastel (11) found the free ACh in whole rat brain to be "less than 1.0γ /gram" and the total ACh to range from 1.0 to 2.9γ/gram.

In the account of experiments to follow, it should be noted that a control rat was nearly always run with each experimental rat and the two extracts assayed on the same heart preparations. This avoided, to some extent, variations resulting from small differences in extraction procedure and assay, which inevitably resulted from having several persons participating in the experiments, thus making differences between experimental animals and controls obvious when a series was not large enough to permit statistical analysis of the data.

2. *Low atmospheric pressure and ACh level of cortex.* Three groups of rats without pretreatment were exposed individually for 1–2 hours to periodic decreases in atmospheric pressure until respiratory movements ceased. They were then decapitated and cortical tissue extracted and assayed for ACh. A total of nineteen rats were exposed to low pressure and twenty normal rats used as controls. The results are summarized in Table 1. It may be noted that the range of individual values for ACh in normal and low pressure rats overlap but in each group, and by both methods of extraction and assay, the average values for rats subjected to low pressure are lower than for a corresponding number of normal rats. The decrease in free or total ACh in the different groups and by the different assays varies from 31 to 52 per cent.

Table 1. Acetylcholine levels of cerebral cortex of rats under conditions of low atmospheric pressure. A. Total ACh by trichloracetic acid extraction. B. Free ACh by cold-Ringer extraction. Summer Series. C. Free ACh by cold-Ringer extraction. Fall Series

		Assays on Venus Heart			Assays on Frog Heart		
No. of animals	Condition	Range	Average	S.E.	Range	Average	S.E.
		AChγ/gm.	AChγ/gm.		AChγ/gm.	AChγ/gm.	
A. 5	Normal	0.9–2.8	1.6	0.32	0.25–1.0	0.46	0.15
5	low pressure	0.4–2.0	1.1	0.29	0.10–0.80	0.28	0.13
B. 9	Normal	0.12–0.60	0.36	0.057	0.13–0.40	0.26	0.04
8	low pressure	0.10–0.28	0.17	0.023	0.10–0.25	0.15	0.02
C. 6	Normal	—	—	—	0.20–0.75	0.35	0.10
6	low pressure	—	—	—	0.13–0.45	0.23	0.09

A statistical analysis of the data of Table 1 shows that only in series B are the differences in the mean ACh values of normal and experimental rats significant, according to the usual method of determining significance. Both sets of assays give differences between the means which are more than twice the standard error of the difference.

In series A and C, which contain too few determinations for statistical analysis, in only two instances were the ACh values of the cortex of low pressure-treated rats the same as those of the normals tested on the same assay preparation. In one of these (rats 31 and 32, Table 2) the same extracts when tested on the Venus heart yielded a lower value for the cortex of the experimental rat. To illustrate this point the data of series A Table 1 are shown in Table 2.

There is little doubt that much of the apparent variation in ACh content of a given normal tissue which we, and others, have observed is due to the variability of the assay preparations. When cortical tissues of two or more normal rats are extracted and assayed on the same test preparation the values obtained are much more uniform than when the same extracts are

Table 2. Individual values for total ACh, (Series A—Table I.)

Assays on Venus Heart				Assays on Frog Heart			
Normal		Low Pressure		Normal		Low Pressure	
Rat	AChγ/gm.	Rat	AChγ/gm.	Rat	AChγ/gm.	Rat	AChγ/gm.
19	2.8	18	2.0	19	1.00	18	0.80
24	1.6	25	1.3	24	0.50	25	0.10
27	1.4	28	0.7	27	0.25	28	0.12
29	0.9	30	0.4	29	0.30	30	0.15
32	1.2	31	1.0	32	0.25	31	0.25
	av. 1.6		av. 1.1		av. 0.46		av. 0.28

assayed on different test preparations. It was, for this reason, that an extract of the cortex of a normal rat was assayed with that of each experimental rat, on one or more test preparations, and when consistent differences were found they were considered significant.

3. *Prostigmine treatment and low pressure.* If the effect of the low pressure in decreasing the level of ACh in the rat cortex were due to the breakdown of ACh by cholinesterase, in the normal functioning of the brain, and failure to synthesize new ACh due to oxygen lack, it should be possible to prevent or reduce the loss by previous administration of an anti-cholinesterase. Since prostigmine is one of the most effective of such agents it was employed to test this idea.

Pairs of rats were injected subcutaneously with 0.1 or 0.2 cc. of a 1:5000 solution of prostigmine (Prostigmin) bromide (Hoffmann-LaRoche)*. The general reactions of the rats to this amount of prostigmine were comparable to those produced by approximately five times as much eserine. One rat was subjected to the same low pressure treatment as in section 2 above, the other used as a control. Ten pairs of rats were so treated. Extracts were made by the cold-Ringer method except that prostigmine was used as an anti-cholinesterase instead of eserine. Assays were made on frog hearts only. The ten control rats kept for 1–2 hours after the injection of prostigmine gave values for free ACh ranging from 0.2 to 0.7γ/gram of cortex with an average value of 0.44γ. The rats receiving prostigmine and the low pressure treatment yielded values ranging from 0.2 to 0.75γ ACh with an average value of 0.43γ/gram. Such close agreement between the controls and the low pressure rats may be quite accidental, nevertheless one would conclude that treatment with prostigmine does prevent the decrease in ACh found in rats subjected to low pressures without previous administration of this anti-cholinesterase.

Observations of the prostigminized rats while being subjected to low pressure failed to reveal any obvious increased tolerance to the low pressure when compared with rats without prostigmine. To check this, a number of rats were subjected to low pressures repeatedly—one day without prostigmine and the following day with. No constant differences in the behavior of the same individual rat could be observed.

4. *Insulin hypoglycemia and ACh level of cortex.* MacIntosh (9) determined the total ACh in whole brains of seven normal mice and obtained values ranging from 1.5–2.6γ ACh/gram. Six mice killed in insulin convulsions gave values ranging from 1.5–1.8γ/gram. However, he concluded that insulin hypoglycemia has no effect on the ACh level of the mouse brain. Cortell, Feldman and Gellhorn (3) performed similar experiments on rabbits, making assays on the eserinized rectus abdominis muscle of the frog. From eight normal rabbits they obtained values for hemispheres ranging from 1.0–2.0γ/gram (average 1.5), and for brain stem, values ranging from 2.0–2.7γ/gram

* Supplied through the kindness of Hoffmann-LaRoche, Inc.

(average 2.3). Six rabbits given insulin gave values for hemispheres ranging from 1.1–1.7γACh/gram (average 1.3) while values for brain stem ranged from 1.9–2.4γ/gram (average 2.1). These authors likewise concluded that there was no effect of insulin hypoglycemia on the ACh level of the rabbit brain. Nevertheless it was considered worthwhile to repeat such experiments on the rat, measuring the free ACh rather than total ACh and using more sensitive test preparations than those employed by MacIntosh and by Cortell *et al.*

Rats were fasted for 16 to 22 hours, always including one night. Some of these were used as controls, others injected with insulin (5 or more units per

Table 3. Free acetylcholine levels of cerebral cortex of rats under conditions of insulin hypoglycemia. A. Summer Series. B. Autumn Series

No. of Animals	Condition	Assays on Venus Heart		Assays on Frog Heart	
		Range	Average	Range	Average
		AChγ/gm.	AChγ/gm.	AChγ/gm.	AChγ/gm.
A. 5	Fasted only	0.30–0.45	0.34	0.20–0.25	0.23
5	30 min. after insulin	0.10–0.16	0.15	0.06–0.15	0.12
7	convulsions	0.05–0.20	0.12	0.03–0.12	0.09
4	coma	0.14–0.20	0.17	0.10–0.20	0.15
B. 10	Fasted only	—	—	0.20–1.0	0.68
7	convulsions	—	—	0.12–0.40	0.28
2	coma	—	—	0.40–0.50	0.45

kilogram). Two series of experiments were performed independently by different persons. In series A some rats were killed 30 minutes after the injection of insulin, some in convulsions and some in coma. In series B seven were killed in convulsions, only two in coma. Extraction was by the cold-Ringer method with eserine.

The results are given in Table 3. That there is a marked decrease in the level of free ACh in the rat cortex as a result of insulin hypoglycemia is apparent. The greatest decrease is seen in the rats killed in convulsions. In the few rats killed in coma there is some apparent return toward normal levels but too few determinations were made for this to be a final conclusion.

The relatively higher values for ACh in both controls and hypoglycemic rats in series B compared with series A was due to a difference in extraction procedure. In B the triturated cortical tissue was allowed to stand longer before centrifuging than in A.

5. *In vitro experiments.* Five rats were decapitated in insulin convulsions and the cortex divided in approximately equal halves. These were weighed and minced finely in sufficient phosphate-Locke solution (11) containing eserine sulphate 1:10,000, to give 100 mg. of cortex per cc. Each lot was placed in a tube from which the air could be evacuated and glucose was

added to give 0.02 M glucose. One tube was evacuated and sealed; the other was left exposed to air. Both lots were incubated at 37°C. for 2.5 hours with occasional shaking. At the end of this time the contents were centrifuged and the clear centrifugate assayed on the frog heart. Values for free ACh of the cortex incubated with air excluded ranged from 0.1 to 0.2γ/gram. Corresponding halves of the cortex incubated in air gave values approximately ten times as great.

This agrees with the observations (11, 15) that synthesis of ACh *in vitro* is an aerobic process and demonstrates that certain precautions must be observed in the measurement of ACh in tissues of anoxic animals. If, in the extraction process, tissues are allowed to remain at room temperature or higher, with air available, a considerable amount of synthesis of ACh may occur if substrates are present, thus restoring the level to a more or less normal amount. It is believed that by carrying out the extraction in the shortest possible time and at a temperature near 0°C this is largely obviated.

Discussion

From the above results it appears that low atmospheric pressure (probably acting through anoxia) and insulin hypoglycemia produce a decrease in the level of ACh in the cerebral cortex of the rat. While earlier investigators (3, 9) concluded that there was no reduction in total ACh in the hypoglycemic or anoxic mouse or rabbit brain, an examination of the data shows that their highest values for treated animals were, in no case, as large as normal values. Therefore it may be concluded with reasonable certainty that glucose and oxygen are important in the *in vivo* synthesis of ACh as well as for *in vitro* synthesis as had been demonstrated by earlier investigators.

The precise role of ACh in the brain is not known; however this finding that the level of ACh is modified by low oxygen and low blood sugar helps in the understanding of certain observations on electrical activity in the cortex. It has been demonstrated (5, 6, 16 and others) that almost any procedure which alters the supply of glucose to the central nervous system, or the oxidation of the glucose, alters the electrical picture in the brain. In hypoglycemia there is a decrease in alpha waves and an appearance of large slow delta waves indicating a loss of excitability. At the time of convulsions the cortex is "silent" or nearly so (4).

The opposite occurs when eserine or prostigmine (or ACh after one of these) is applied to the cortex. Miller, Stravraky and Woonton (13) applied eserine to the rabbit cortex and noted the reduction in amplitude of slow waves and the appearance of small fast waves. ACh after eserine in the rabbit and cat caused the disappearance of slow waves and the appearance of spikes of high amplitude and frequency. Chatfield and Dempsey (2) applied ACh after prostigmine, to somesthetic, auditory and motor areas of the cat cortex and noted increased spontaneous activity and the appearance of 5–10/sec. spikes followed by 20–30/sec. low voltage potentials. This

suggests that the decrease in ACh resulting from anoxia or hypoglycemia is responsible for the decreased level of excitability of the cortex.

Summary

1. Several methods of extraction and assay of free and total ACh in the cerebral cortex of the normal rat are compared.

2. Subjecting rats to low atmospheric pressure for 1 to 2 hours is shown to decrease the level of free or total ACh in the cortex by approximately one third to one half.

3. Administration of prostigmine before low pressure treatment prevents a decrease in free ACh in the cortex.

4. Insulin hypoglycemia results in a greater decrease in free ACh than that produced by the low pressure treatment.

5. It is suggested that the decline of free ACh may account for the decrease in excitability of the cortex under conditions of anoxia and hypoglycemia.

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ACCOMMODATION AND AUTORHYTHMIC MECHANISM IN SINGLE SENSORY FIBRES

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THE TECHNIQUE for measuring accommodation directly in relation to the discharge of impulses, developed in this laboratory by Skoglund (21), makes it possible to correlate a number of properties of nervous activity with the accommodation curves. The latter serve as reference point in the analysis which also by this method is shifting emphasis from measurements of threshold variations to inspection of the discharge as such and measurements of its properties. A further improvement upon Skoglund's work is introduced in this paper by the use of micro-electrodes, placed directly on the sensory roots in order to isolate single fibres in nerves electrically stimulated with linearly rising currents. This technique is here used for the purpose of studying the properties of the autorhythmic mechanism in sensory fibres of different accommodative resistance.

Bernhard, Granit and Skoglund (6) proposed as a working hypothesis that the electrotonic potential, seen in spinal roots by Barron and Matthews (5) and studied in the optic nerve by Bernhard (7, 8, 9) serves as exciting for the autorhythmic mechanism in the nerve itself. From this and allied points of view we need more information about the properties of the rhythmic discharge caused by stimulation with slowly rising currents imitating possible generator potentials conducted electrotonically from the axon hillock down the fibres.

TECHNIQUE AND PROCEDURE

Stimulation. The stimulating device, built by the physicist of this laboratory, Mr. K. T. Helme, has been described in detail by Skoglund (21). The apparatus delivers linearly increasing currents of strictly controllable gradient and strength through the anode circuit of a valve. This stimulator is connected so as to shift the one beam of a double cathode ray in proportion to the rate of rise of the stimulating currents, the other beam being used for simultaneous records of the discharge in the nerve through a condenser coupled amplifier (see the figures of this paper). Strength and gradient are independent variables. The stimulus was driven up to a certain strength at a certain rate, then left at plateau height for some time, and finally allowed to drop back at a rate of fall corresponding to its rate of rise. If the plateaus were brief, the stimulator was operated iteratively by a sweep circuit. With longer plateaus it was necessary to start the stimulator manually. Plateaus from a few milliseconds to some 60 seconds were used.

Preparation. The spinal cord of decerebrate cats was laid bare and the animals slightly lifted up in the preparation box by a specially designed clamp gripping firmly around one thoracic and one sacral spinous process and rigidly fixed to a heavy stand. These precautions are necessary for the sake of the micro-electrode. Without a clamp on the vertebrae themselves each respiratory movement is accompanied by considerable excursions of the preparation under the electrode. Fine adjustment of the latter was achieved with the aid of a micromanipulator. Well chlorinated silver-silverchloride electrodes were used for stimulation. In order to differentiate between nerves from the cutaneous and the muscular end organs these electrodes were either placed on the saphenous nerve or on twigs from the

popliteal nerve where they enter the fleshy portion of the muscles below the knee. Sometimes the whole sciatic stem was used.

The micro-electrode was of the type used in this laboratory (14) for work on the retina and consisted of a fine platinum wire insulated by glass. It is pressed vertically against the dorsal roots not far from the point where they enter the cord. Some fibres are then found to be spontaneously active, others silent, some again are incited to activity by the pressure of the micro-electrode. Spontaneous discharges may or may not be accelerated by the stimulus. When relatively thin muscular branches are stimulated it is difficult to find an active fibre. It is also easier to locate a diffuse discharge in response to stimulation than to succeed in isolating a single spike. It is possible, in cases when a large spike suddenly turns up in perfect isolation, that it represents a certain amount of synchronized activity. Still, the discharges look and behave like so-called single units. Their often less strict correspondence with the all-or-none law can be explained by the influence of the discharge in adjacent elements.

A time signal interrupts the beam for marking the stimulus. Dependent upon what is being pictured this beam is interrupted at a rate of 50 or 500 per sec.

Results

All-or-none nature of response. Only if several active fibres are lying under the electrode can the dorsal root reflex (16, 22), conducted centrifugally from the spinal cord, be a source of error. However, the latent period of this discharge is much longer than that of the direct centripetal volley. But, when the micro-electrode is used, the chance of finding a centrifugally conducting fibre is practically *nil*. It is difficult to isolate a directly excited single spike.

In Fig. 1 are illustrated the effects of a variation in (i) strength of stimulus at constant rate of rise and (ii) length of plateau at constant strength

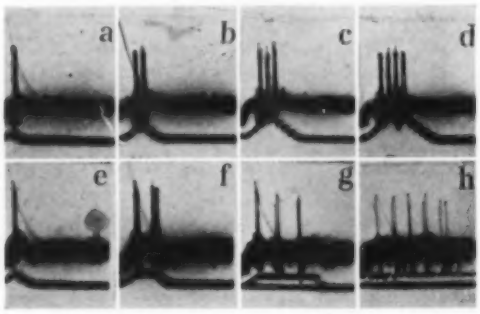


Fig. 1. Superimposed pictures of several sweeps with the cathode ray. Form and strength of stimulus on lower cathode ray. Time in 500/sec. From a to d stimulus increases in strength at constant rate of rise, a, threshold, b, 2.4 rheobases, c, 3.7 rheobases, d, 5.0 rheobases. From e to f lengthening of duration of plateau at constant strength of the stimulus and constant rate of rise.

and rate of rise. In both cases the same stimulus pattern was swept several times across the screen of the tube. In none of the later pictures has this particular technical arrangement been used. Figure 1 is reproduced to show the all-or-none manner in which impulses are added up to a rhythmic discharge. With less perfect isolation the spikes diminish when strength or gradient is diminished. Both variations, in strength from a to d, and in plateau length from e to h, show that spikes are added with such regularity that the whole process can be swept across the tube several times without any other effect than a slight increase in width of the spikes, indicating minor variations of latent period. Our technique is thus sufficiently discriminative for the purpose for which we intend to use it.

Accommodations curve. In order to plot accommodation curves (21) the actual stimuli of different rates of rise are drawn in a co-ordinate system in which the abscissae accordingly are rising times and the ordinates strength. These values are obtained directly from the photographed curves (see figures) in which the final plateau level of current always was checked by a milliammeter in the circuit. Thus one does not merely rely on the amplified deflexion of the "stimulus-beam" of the cathode ray. With a large range of stimulus intensities tested, it is often necessary to alter the degree of amplification in order to keep the beam within the proportionality range of the instruments.

The various stimuli having been inserted into the co-ordinate system, the moment of appearance of the *first* spike is marked on the curve for each linearly rising stimulus. The points so obtained are joined to a curve (see Fig. 2) which accordingly illustrates the strength to which it has been neces-

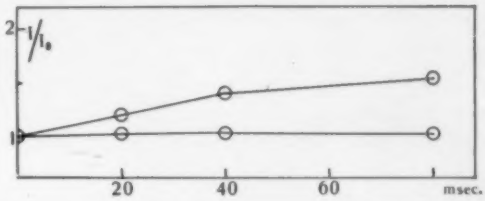


FIG. 2. Average accommodation curve for muscular afferents (upper curve) and for the saphenous (lower curve). Ordinates multiples of rheobasic strength (I/I_0) (see text).

sary to drive the slowly rising current at each particular gradient in order to elicit *one* impulse, the first of a series, or a single spike if others do not follow. For rapidly rising stimuli the conduction time must be substracted.

The graph so obtained is of the same type as the one introduced by von Kries (18) and also used by Hill (15) and his collaborators. Depending upon the particular problem in view, strength of current (I) may be given in milliamp. or in multiples of rheobasic strength (I/I_0). The inverse value of the rate of rise of the initial rectilinear portion of the curve corresponds to Hill's constant λ. However, Skoglund's direct method of measuring accommodation and plotting the curve from photographed responses eliminates a number of errors and difficulties inherent in the methods based on observation of a threshold muscle contraction as "constant" index (6, 21) and makes it possible to isolate single fibres, not to mention the advantage of also being able to study sensory nerves by the direct method.

In Fig. 2 are plotted the average accommodation curves for muscular afferents and the purely cutaneous saphenous nerve (A fibres). The latter curve hardly rises at all above the rheobase. The muscular afferent also has relatively little accommodation, compared with motor nerves (12, 21). Breakdown of accommodation follows at about 1.5 rheobases. Hill's constant λ was about 150–200 msec. with muscular afferents and approaching infinity with the saphenous. These differences, as we shall see, are large enough to reappear mirrored in the properties of the autorhythmic mechanism of the nerves.

Two phases of rhythmic discharge. "Silent period." It has been pointed out previously (11, 21) that the rhythmic discharge can be divided into two phases, initial spikes of high frequency and a later prolonged discharge. We have now an opportunity of finding out whether these phases occur in a single element or represent different fibres. From this point of view Fig. 3 is of particular interest. The rate of rise is constant and the final strength of the current varies from a to f. Record g is from another experiment.

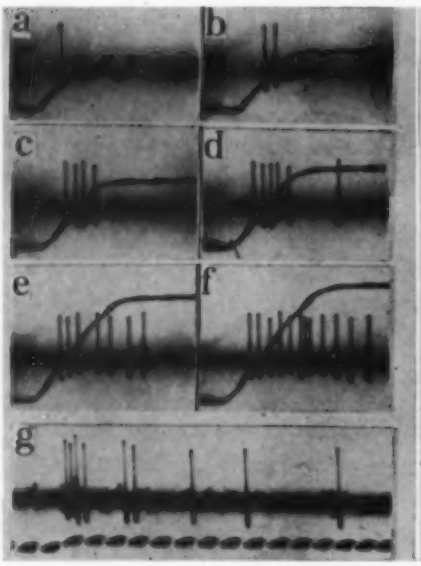


FIG. 3. Muscular afferent. Stimuli rising from a to f at constant rate of rise to greater strength: a, 1.4 rheobases, b, 1.6, c, 2.0, d, 2.4, e, 3.2 and f, 4.0 rheobases. Record g from another experiment illustrates "silent period."

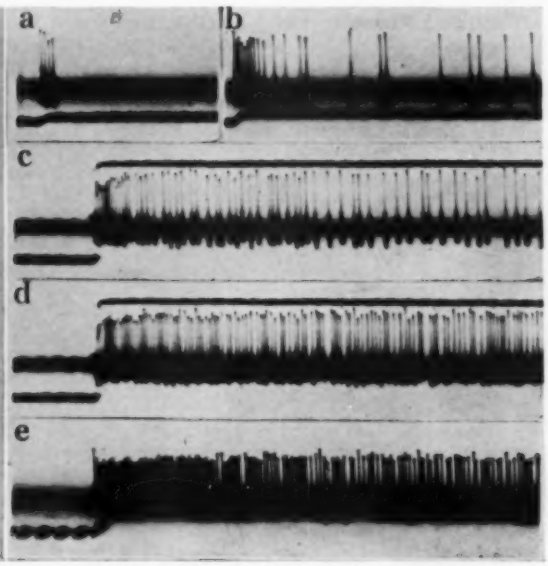


FIG. 4. Muscular afferent. Strength of stimulus increases from a to e (see text). The plateau frequencies are: a, 0, b, 75, c, 140, d, 275 and e, 450 per sec. These are counted after about 20 msec. of stimulation. Time in 50/sec.

The development of the initial phase of the discharge is seen in the records a to d. Continuation of the rising phase of the current is accompanied by the birth of new impulses. In record d, however, there follows after a silent period the first impulse of the later plateau-phase of the discharge. Still stronger stimuli, as in e and f, do not lead to a silent period because the threshold for the plateau-phase is then reached during or just after the rising portion of the stimulus so that it becomes submerged into the initial phase. With linearly rising stimuli the occurrence of a silent period between the two phases of the discharge is left to chance but it could, no doubt, be produced regularly if with our apparatus it were possible to decrease the rate of rise of the stimulus somewhat at the top. In record g the silent period is very prominent.

Previously we (6) have defined as "breakdown of accommodation" the fact that at a certain strength of current its strength alone rather than its rate of rise becomes significant (see Fig. 2) and pointed out that this intensity region is recognized by the flattening of the accommodation curve accompanied by iterative firing. The plateau discharge belongs to this late portion of the accommodation curve. Consequently this is the most important phase of the autorhythmic mechanism in sensory nerves which have low accommodative resistance. In the saphenous nerve it sets in practically as soon as the current has risen a little above rheobasic strength; in muscular afferents there is already a definite initial phase. But on the whole our preparation is more suitable for studying the plateau-phase. Motor nerve should be used for the initial phase. Gradient is there relatively more important.

In sensory nerves the initial phase seems to share with the plateau-phase the property of being more dependent upon current strength than upon its

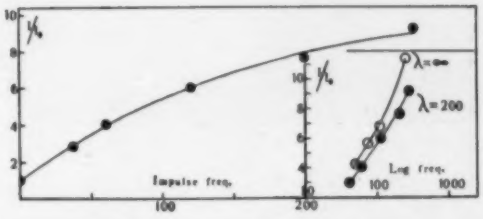


FIG. 5. Muscular afferent. Current strength in multiples of the rheobase as ordinates against plateau frequency of the discharge as abscissae. *Inset.* Same ordinates against log frequency in order to compare a saphenous nerve with a muscular afferent.

gradient. The range of variation in the frequency of the discharge is far greater during the plateau-phase than in the initial phase.

Plateau discharge and current strength. It is known that in general the frequency of the repetitive discharge is greater for stronger stimuli (12, 13, 17, 21). In Fig. 4 is illustrated the effect of current strength on a single muscular afferent with $\lambda = 200$. On account of the large intensity range it has been necessary to use different degrees of amplification of the stimulus-beam of the cathode ray so that the deflexions are not proportional to current strength. In a, at 2.1 rheobases, there is merely the initial phase, in b, at 3.9 rheobases, the plateau-phase is well developed. In c the strength is 5.8 rheobases. The initial phase is from the very first record activated at a frequency high enough to cause diminution of the size of spikes. In d, at 9.7 rheobases, the plateau-phase is activated at a frequency leading to a relatively extended depression of spike size, and in e, at 15.0 rheobases, the spikes are subnormal for the whole period illustrated. The maximal frequency is at about 450 per sec.

In Fig. 5 the quantitative relation between spike frequency and current strength in multiples of rheobasic strength (I/I_0) is illustrated. In the inset the abscissae are log frequency and the strength-frequency relation has been compared for a muscular afferent and a saphenous. The difference between these two curves does not exceed the range of variation in different experiments. The asymptote of the curves is found to begin in the frequency re-

gion 400–500. We conclude that variations in accommodative resistance within the limits of these experiments have no definite influence on the strength-frequency relation.

Adaptation and accommodation. By adaptation is meant the fact that the frequency of the discharge gradually sinks during prolonged stimulation at plateau height. Figure 6 illustrates different stages of this process during the course of stimulation with a current 5.8 times the rheobase. This nerve

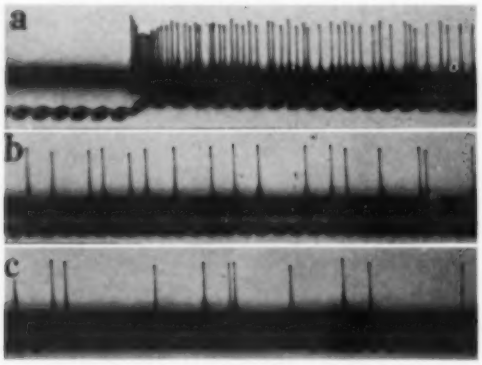
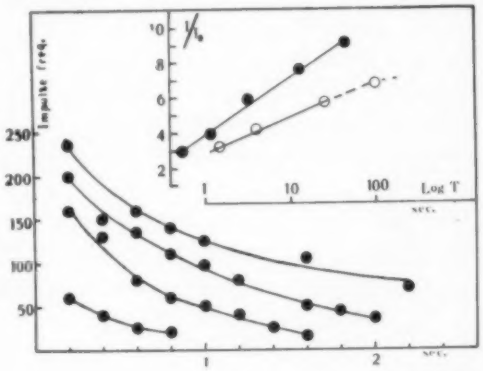


FIG. 6. Muscular afferent. The course of adaptation to plateau strength of a stimulus 5.8 times the rheobase. Record begins in a with about 250 impulses per sec. In b, after 2.5 sec., it has fallen to 35/sec., in c, after 5.0, sec., to 18/sec.

is a muscular afferent. With the saphenous we have often kept the stimulus at plateau height for 60 sec. and still seen a discharge of considerable frequency. But in nerves with some accommodation the frequency much sooner

FIG. 7. Muscular afferent. Course of adaptation for stimuli of different strength in terms of frequency of discharge as ordinates against duration of stimulation as abscissae. *Inset.* Comparison of a muscular afferent (filled circles) of $\lambda = 200$ with a saphenous (open circles) of $\lambda = \infty$ with respect to the total adaptation time T for the rhythmic discharge at strengths of stimulation given as ordinates in multiples of the rheobase. Log T on the abscissa. Stimulation has been continued until the rhythmic discharge has stopped.



reaches zero. In such cases the total adaptation time (T) can be measured.

A set of curves illustrating the decline in frequency from different levels of excitation (I/I_0), defined by the initial plateau frequencies, is found in Fig. 7. The abscissae show the duration of the plateau stimulus. The nerve is a muscular afferent. In the inset of Fig. 7 multiples of rheobasic strength (I/I_0) are plotted against the logarithm of the total adaptation time (log T), *i.e.* the time from the first to the last impulse of the discharge caused by

a certain stimulus. Here, as in all our experiments, the total adaptation time is a function of the accommodative resistance. The steeper the rise of the accommodation curve, the steeper also the rise of the curve in the I/I_0—log T graph, the shorter consequently the adaptation time for a given multiple of rheobasic strength.

This difference is well brought out in the inset of Fig. 7 by the comparison of the muscular afferent of $\lambda = 200$ with the saphenous of a λ approaching infinity. It is further emphasized by an interesting experiment in which the accommodation gradually rose while we succeeded in keeping the same fibre for several hours under the micro-electrode. Hill's constant λ was about 200

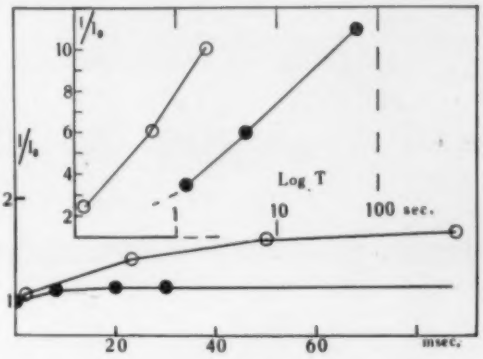


FIG. 8. Accommodation curves for a muscular afferent in the beginning (filled circles) and at the end (open circles) of an experiment (see text). *Inset.* Plot of log T (abscissae) against multiples of rheobasic strength (ordinates) as in inset of Fig. 7. Curve to the left thus belongs to nerve giving late accommodation curve ($\lambda = 85$), curve to the right to nerve giving early accommodation curve ($\lambda = 200$).

in the beginning of the experiment and around 85 some hours later. The two accommodation curves from which these approximated constants were obtained (from the early portion of the curve, as always) are shown in Fig. 8. In the inset of the same figure are plotted the logarithms of the total adaptation times (log T), just as in the inset of Fig. 7. The plot shows that the shortening of the adaptation time and the consequent steeper rise and shift of the I/I_0—log T curve actually is a function of the accommodative resistance of the active fibre and not due to differences in structure between muscular afferents and the saphenous, of a character unconnected with accommodation. Both curves here refer to the same single muscular afferent fibre in different states of accommodation.

Adaptation and cathodal depression. Turning now to the original record of the experiment, evaluated above in Fig. 8, we find in Fig. 9 the late stage of it, characterized by $\lambda = 85$, which should be compared with the records in Fig. 6 from the early stage ($\lambda = 200$). In Fig. 6, at 5.8 rheobases, there was still a plateau discharge present 5.0 sec. after initiation of the rhythmic activity. In record b of Fig. 9, at 6.0 rheobases, the discharge stops after a little over half a second. It does not last very much longer when the strength is increased in c to 9.0 rheobases. The reason for this becomes evident when the current is further increased, to 14 rheobases in d. The whole plateau discharge is now inhibited and possibly also the last portion of the initial phase. This is the cathodal depression, noted by Schiff (20), then seen

by several others, and well analyzed by Werigo (23). It is often nowadays (17, 19) called Werigo's cathodal depression. This block is a decisive factor in "adaptation" and probably determines adaptation time (log T) at all strengths of current, though visible as a complete inhibition of the discharge only at higher levels of stimulation intensity, provided that the accommo-

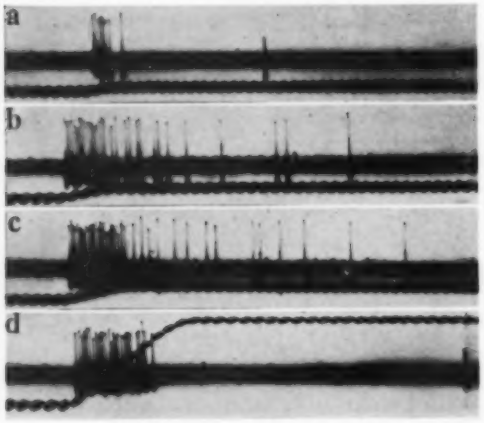


FIG. 9. Record belongs to muscular afferent giving upper accommodation curve of Fig. 8. It illustrates time course of adaptation for increasing plateau strengths and constant rate of rise: a, 2.0, b, 6.0, c, 9.0 and d, 14.0 rheobases.

dative resistance is sufficiently high. In nerves with little accommodation the cathodal depression must be weakly developed or so late as to have fallen outside our longest observation times (around a minute). With motor nerves Skoglund (as yet unpublished work) finds it regularly and has been studying the phenomenon in this laboratory. Its connexion with accommodation is shown by the fact that it here also turns up in a sensory nerve as soon as accommodation has risen a little above the commonly obtained values. In Fig. 4, record e, there was no cathodal depression even at 15.0 rheobases.

"*Intermittent conduction.*" In studying the centrifugal discharge through the *dorsal* roots in response to adequate stimulation of different afferents Barron and Matthews (4) noted that it sometimes was split up into intermittent groups of impulses. Such intermittency was held by them to be due to temporary blockage in the spinal cord, caused by cord potentials conducted electrotonically to some critical point in the passage of the excitatory disturbance. However, in our experience a similar phenomenon is found also in peripheral sensory nerves.

Intermittent spontaneous discharges of great regularity are seen every now and then with an afferent root under the micro-electrode. The source of these spontaneous intermittent discharges is the cut end of the nerve. They disappear if a drop of a cocaine solution is applied onto the severed region of the afferent nerve and thus belong to the "grouped injury discharges" described by Adrian (2). But there are also cases in which the nerve is silent and reacts to the plateau stimulus with an intermittent discharge.

The activation of such a dormant tendency to intermittent conduction is shown in Fig. 10. The stimulus seems to facilitate a process on the verge of activity. Similar observations have been reported by Erlanger and Blair (11). Barron and Matthews have emphasized the significance of their own observations for central inhibition. Like most central phenomena, this also seems to have its peripheral counterpart. Barron and Matthews' explanation could be adapted to fit our case but there is little reason to theorize here about observations which have been wholly unsystematical.

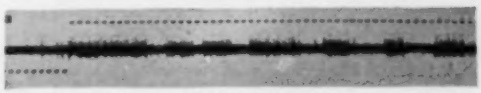


FIG. 10. "Intermittent conduction." Stimulation of muscular afferent elicits discharge which soon is split up into regular groups.

DISCUSSION

Adaptation time, accommodation and cathodal depression. Hill's theory (15) explains the occurrence of rhythmic discharges to constant currents by the assumption that the "local potential" V for a certain time T remains above the "threshold" U. In the words of Katz (17): "during a certain time T, therefore, V will be greater than U, and throughout this time repetitive response might be expected to occur, at intervals determined by the refractory period." Stronger stimuli increase the frequency by eliciting impulses earlier in the refractory period.

The relation between this time T (which here has been called total adaptation time), the constant λ, and stimulus strength I/I_0 has also been given by Katz. It is $T = \lambda \log\hat{e}\, I/I_0$. In frogs (whole nerve) he finds good argeement between experiment and theory. The reason for this may be the limited range of intensities tested. It is immediately seen that in our experiments with a value for λ around 200 msec. T may be of the order of 1000 msec., for I/I_0 around 3–4. The formula does not therefore fit our case. It may nevertheless be of some interest to compare the curves for the sensory fibre (Figs. 8 and 9) in which λ was 200 in the beginning and 85 at the end of the experiment. Calling the adaptation times T_{200} and T_{85} for $\lambda_1 = 200$ and $\lambda_2 = 85$, it is clear that on the theory

$$T_{200} - T_{85} = \log\hat{e}\, I/I_0\ (\lambda_1 - \lambda_2),$$

the difference between the two constants being 115.

The right and left members of this equation are compared in Table 1 for different values of I/I_0.

Table 1

I/I_0	115 $\log_e I/I_0$	$T_{200} - T_{85}$
10	262	38.000
6	206	4.400
4	160	1.500
2 extrapolated	79	0.230

The total adaptation time is thus of an order of magnitude which suggests, by decreasing with I/I_0, that quantitative agreement with the theory in sensory nerves only can be expected below the double rheobase. Nevertheless the theory has been a valuable instrument in the experimental analysis.

However, accommodation appears in an important function as common denominator for the general resistance of the nerve to impulse production. The greater this resistance, the more developed the cathodal depression, and the shorter the adaptation time which probably, as stated, is determined by the development of the Schiff-Werigo inhibition. This is an instance of what is meant by our statement in the introduction that accommodation serves as reference point in the analysis.

Whether this late cathodal depression can be equated with the early cathodal depression, studied by Erlanger and Blair (10, 12), remains to be found out in work with motor nerve where accommodative resistance is very much better developed. But it is clearly important that in their work too, accommodation, the early cathodal depression and the degree of repetitiousness are connected in the manner in which they now appear connected here (cf. also 21) despite the different mode of attack. Early cathodal depression is synonymous with accommodation as measured by Erlanger and Blair by a method based on testing the cathodal excitability with a shock technique.

Autorhythmic discharge. The fact that the sensory nerves, which are activated individually by isolated peripheral structures with limited energy resources, possess little accommodative resistance by comparison with motor nerves (12, 21) is in agreement with our hypothesis that the autorhythmic mechanism of these nerves can be put into operation by generator potentials carried down electrotonically along the nerve in simple end organs probably by direct chemical excitation of the nerve fibre. From this point of view it is interesting that particularly low accommodation and little adaptation is found in a cutaneous nerve such as the saphenous in which, on the theory, the autorhythmic mechanism would be started by energy released by such weak stimuli as touch or the bending of a hair. However, the degree of adaptation is clearly determined by the end organ and not by the mechanism in the nerve, as directly demonstrated by Adrian, Cattell and Hoagland (3) for cutaneous organs. In the nerve, adaptation of the autorhythmic mechanism is less marked in the saphenous, and clearly noticeable in the muscular afferents whereas the adaptabilities of the corresponding end organs are the other way round (1).

The fact that "gradient" is of relatively little significance in sensory nerves but highly important for many sense organs would seem to throw some light on the differentiation of such sense organs into a primary apparatus for picking up the energy of the adequate stimulus and a secondary generator mechanism firing the nerve. Sufficiently strong stimuli may, however, cause a "gradient" also in the generator mechanism and lead to a discharge interrupted by a "silent period" (initial phase followed by plateau-phase).

Considering the difference in accommodation between motor and sensory nerves it is clear that in reflex activity the transition from the one system to the other suggests interesting possibilities for selection on the basis of differences in accommodative resistance in the internuncial and output channels of the central nervous system.

Summary

The repetitive discharge in response to slowly rising linear stimuli has been recorded with the aid of micro-electrodes from cutaneous and muscular afferents. Single fibres could be isolated by placing the micro-electrode on the dorsal roots. Stimulus form and nerve response are pictured simultaneously with the aid of a double cathode ray oscillograph on the same film.

By this method it is possible to measure the sensory accommodation curves directly and at the same time correlate them with the properties of the iterative discharge.

There is little if any accommodation in *n. saphenous*, representing cutaneous afferents (Hill's constant λ approaching infinity). For different muscular twigs of *n. popliteus* the values for λ range from 150 to 200 msec.

The autorhythmic discharge caused by the slowly rising stimuli consists of an initial phase during the time the stimulus rises and a later plateau-phase when the stimulus has reached a certain plateau level of strength. These two phases may be separated by a "silent period."

The plateau discharge is characterized by a frequency which increases with stimulus strength. The strength-frequency curve for single fibres is illustrated in Fig. 5. It is independent of the accommodative resistance of the nerve.

The total adaptation time (from first to last impulse) of the plateau discharge is a function of accommodation and of stimulus strength and decreases when the accommodative resistance increases or stimulus strength decreases. These relations are illustrated quantitatively for nerves of different accommodation in Fig. 7 and 8.

Strong stimuli continued on plateau height inhibit the discharge (Schiff-Werigo's cathodal depression), provided that the nerves possess good accommodative resistance. It is suggested that the total adaptation time is largely determined by this factor.

The slowly rising stimulus sometimes causes a rhythmically grouped discharge instead of a continuous flow of impulses.

We are indebted to the Rockefeller Foundation for a grant to this laboratory.

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THE RELATION OF AREA 13 ON ORBITAL SURFACE OF FRONTAL LOBES TO HYPERACTIVITY AND HYPERPHAGIA IN MONKEYS*

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HYPERACTIVITY is one of the principal objective consequences of bilateral prefrontal lesions; it has been described for representative animals from the rat to the monkey. But it is often not realized that hyperactivity is an inconstant result of prefrontal lobe lesions (4), and no adequate explanation of the inconstancy of its appearance is at hand. The time of onset of heightened activity is also exceedingly variable, being often delayed as much as three weeks and sometimes preceded by a period of depressed motility not ascribable to paresis. Neither is it known whether any component area of the prefrontal lobe is especially significant in the production of hyperactivity; some believe that prefrontal lobectomy alone gives maximal hyperactivity while others have designated special areas. On none of these points is the literature entirely unequivocal.

Bianchi (6) and Franz (7) made passing mention of hyperactivity in their protocols of monkeys subjected to prefrontal lesions but neither writer includes hyperactivity in summarizing frontal lobe symptomatology. Both note that some monkeys became hyperactive while others appeared dull, indifferent or somnolent. Jacobsen (8) is clearly to be credited with recognizing that hyperactivity is one of the principal symptoms of prefrontal destruction in the monkey; 4 of the original series of 5 monkeys were hyperactive and in a later series (9) 2 of 3 monkeys were hyperactive. But only in two recent studies has altered activity been objectively and quantitatively recorded and the lesions made with modern intracranial surgical techniques.

All of Richter and Hines' (21) series of 4 monkeys in which the prefrontal pole or cortex was removed were hyperactive. With respect to localization within the prefrontal cortex, they conclude that activity is particularly controlled through area 9 and also by the striatum. However of the 2 animals in which area 9 was removed bilaterally only one showed really marked increase of activity (4). They further state that unilateral and bilateral ablation of areas 8 or of 10–11–12 had little or no effect on activity. Contrary to this, Kennard and Ectors (10) reported that unilateral or bilateral ablation of area 8 in monkeys definitely produces hyperactivity. Kennard, Spencer and Fountain (11) later concluded that bilateral and separate ablation of

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area 8, of area 9, or of areas 10–11–12 produced some increase in activity but always short of that produced by removal of the whole frontal association area. The figures in both these papers indicate that the hyperactivity was usually delayed in onset and Kennard *et al.* emphasize the variability in the time of onset. Others have observed hyperactivity in the monkey (16, 23).

For the cat the situation is roughly similar. Barris (3) failed to observe hyperactivity following bilateral removal of the rostral portions of the neocortex but rather described symptoms of a cataleptic nature. Magoun and Ranson (15) were unable to confirm this latter finding but instead obtained hyperactivity. Langworthy and Richter (13) noted increased spontaneous activity in 9 of 10 animals in which they ablated the electrically excitable motor cortex, the premotor cortex and possibly a small tip of the corpus striatum. In earlier experiments, Langworthy and Kolb (12) destroyed various regions of the frontal cortex in 25 cats. Ten of these became hyperactive and the critical area was ventrolateral to the motor area. In the rat, Richter and Hawkes (20) found that unilateral as well as bilateral removal of the frontal pole (cortex and tip of the striatum) consistently increased activity. On the other hand in Beach's (4) series of 9 rats with lesions of the anterior cortex, only 6 became hyperactive and 3 became hypoactive. Striatal lesions were without consistent effect (5).

While only the most recent workers have recorded hyperactivity objectively, the phenomenon is so specific and outspoken, at least in monkeys, that it could scarcely be missed. The inconstancy of hyperactivity is therefore not likely to be due to any difficulty of observation. It is more probable that some variation in the lesion is responsible and this could be either an omission of a critical area or the inclusion of some region productive of apathy or hypoactivity. A consideration of the types of frontal lesions studied with respect to hyperactivity led us to the belief that the variable factor from lesion to lesion lay in the amount of destruction of the orbital surface of the frontal lobe. This present series of focal ablations on this surface indicates that area 13, which has recently been delineated cytoarchitecturally by Walker (22) is of especial importance in the production of hyperactivity.

METHODS

Macaca mulatta monkeys (T.T. Series, Nos. 13, 14, 16, 21, 24) were used exclusively. The operations were performed aseptically under intraperitoneal sodium amytal anesthesia. A horse-shoe shaped skin-flap was reflected frontally and a transfrontal osteoplastic bone-flap was hinged on the temporal muscle. The dura was incised along the margins of the bone-flap, the superior longitudinal sinus was ligated and sectioned at its anterior extremity and the falx and the olfactory nerves were divided. Shifting the animal to the supine position allowed the brain to fall well away from the orbital plate. The pia-arachnoid was coagulated with the Bovie unit along the margins of area 13, which were laterally the frontomarginal sulcus, medially the orbital sulcus, posteriorly the lateral olfactory striae; anteriorly the orbital gyrus was transected at the level of the bifurcation of the orbital sulcus. The bloc extirpated is roughly one sixth of the orbital face of the frontal lobes.

Activity was recorded pre- and postoperatively in the apparatus devised by Kennard, Spencer and Fountain (11). This is an oblong cage 4 ft. by 1.25 ft. and 1.25 ft. high, set

over a freely movable galvanized iron pan, one end of which rests on a pneumatic pad. The pad is connected with a tambour which records upon a long-paper kymograph in ink. Any movement toward or away from the end resting on the pad causes an excursion of the pen and the height roughly reflects the extent of movement. This apparatus, unlike that of Richter and Hines (20) is only semiquantitative but does record graphically the spatial and temporal pattern of the activity; it is free from the objection of emphasizing a turning pattern of locomotion. Records were made before feeding, usually in the morning; they were of 3 hours' duration, and were obtained on 3 consecutive days before operation. Activity was recorded immediately before operation and on the 1st–5th postoperative days and 2, 4, and 8 weeks postoperatively.

Results

Activity. Bilateral ablation of area 13 of the orbital surface of the frontal lobe in one stage has produced marked hyperactivity of immediate onset in 4 consecutive monkeys. The postoperative activity has certain characteristics which make it easily recognizable. It is a change in quality and not merely a quantitative increase of normal spontaneous activity. Thus an operated animal at a time when hyperactivity is minimal is distinguishable from a normal monkey which might display an equal amount of total activity. Of first importance is that the hyperactivity is manifested clearly only in locomotor activity, other forms of movement tending to be restricted, especially in the first postoperative week. Conspicuous is the methodical, stereotyped character of the "pacing" which constitutes the main motor performance of these animals. In a square cage this tends to be about the perimeter of the cage and not consistently in one direction; in a long, narrow cage it it reminiscent of the pacing of a caged lion though much faster except in the first postoperative days. Though the total amount of time spent in walking is greatly increased, the pacing is not strictly incessant for it tends to occur in "bouts" of walking punctuated by rests or pauses. Even when the periods of walking are short and the pauses are long as in the first postoperative days there is a characteristic sterotypy of the operated animals' walking that can easily be seen in the activity records.

The record of a normal monkey (Fig. 1 and 3) reflects a varied pattern of movement about the cage. Rarely is seen more than one or two regularly spaced excursions representing rhythmically repeated "round trips." Periods of activity, periods of slight activity and periods of rest alternate in an infinitely varied pattern. Little evidence of such random activity can be seen in the records or cage activity of area 13 animals. The activities of the operated monkey are largely reduced to two: either methodical walking or quiet resting. The record rarely shows a single translocation from one end of the cage to the other followed by a rest or random activity (denoted by small excursions superimposed on a change in the base line); once walking is initiated it tends to continue at a regular rate for many round trips. It is as though an attempt to move from one end of the cage to the other initiates perseverative walking. Such bouts of walking may last as long as the test period of 3 hours. The pauses too appear not to be interrupted by small random movements (small excursions of the recording pen) that often mark

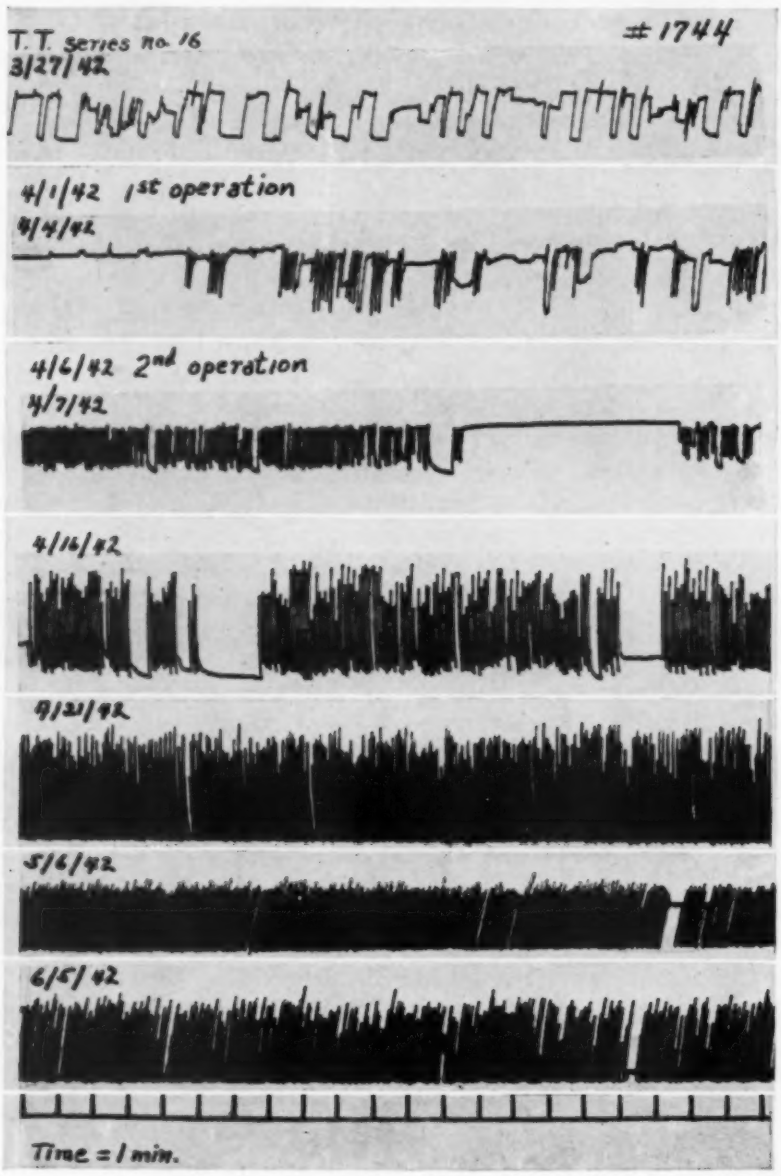

FIG. 1. Records showing development of hyperactivity after bilateral ablation of area 13 (second operation) subsequent to ligation of superior longitudinal sinus at its anterior end. Note rapid onset of hyperactivity after second operation and that maximal hyperactivity was reached in two weeks.

the pauses in the records of normal animals. Thus the effect of area 13 lesions is not only to increase the amount of activity but also to simplify the complex interplay of postures and movement.

Onset and persistence of hyperactivity. Hyperactivity has consistently been observed as early as the first postoperative day. At a time when the effects of surgical shock, anesthesia, etc. were still apparent, characteristic pacing was observed; it is slow and frequently interrupted by periods of immobility but does not differ in quality from maximal hyperactivity. A record of activity made on the first postoperative day is shown in Fig. 1. The amount of time devoted to pacing and the rate of walking increases to a maximum in the 3rd or 4th week. In the second postoperative month total activity diminishes somewhat. However, as can be seen in Fig. 2 there may be little or no decrease, and in no animal was there a return to the preoperative activity level. Two animals (No. 16 and 24) have been kept for more than 6 months and continued to exhibit striking hyperactivity; the alteration in behavior apparently is permanent. The negative symptoms described in the next section, unlike hyperactivity, are most marked in the first and second weeks.

Negative symptoms. Behavior deficits, at least in the first week after operation, are almost as striking as the overactivity, though difficult to catalogue. There is a definite reduction in emotional expression. Fear seems reduced. Aggressive behavior consisting of opening the mouth with jutting forward of the head and shoulders and fixed gaze so typical of the normal macaque is absent for some days or weeks; and this appears to be permanent in some animals. Unmistakable too is the lack of response to human presence; instead of watching the observer intently as the cage is approached the operated monkeys when not pacing tend to sit gazing into the distance with a blank expression. Later after the operation the animals become more reactive, perhaps over-reactive, to the observer but excitement still produces an abnormal response, which is to accelerate the rate of running (Fig. 2). This seems to take the place of the usual fear or aggressive responses. The distractability emphasized by other observers could not be identified. At first food tends to be neglected. When offered a piece of fruit the animal reaches for it but allows it to slip through the hand; the empty hand is often brought to the mouth. Or a bite or two is taken and the food then allowed to fall to the floor. Such behavior persists only for a few days after which the animals eat well.

Controls. The operative technique used involves ligation of the superior longitudinal sinus at its anterior end and some retraction on the tip of the frontal pole. To control the possibility that a generalized prefrontal damage arising from these procedures rather than ablation of area 13 causes the hyperactivity, the following experiments were performed. In one animal (T.T. No. 16) a "dummy" operation consisted of the usual procedures to and including ligation of the longitudinal sinus and severance of the falx. This appears from the activity record (Fig. 1) to have increased activity slightly but neither hyperactivity nor any other departure from the normal was detected in cage behavior. In two additional experiments the sinus was ligated as a part of other procedures without any sign of hyperactivity. To

control the factor of incidental damage to the frontal pole, in a second animal (T.T. No. 21) the frontal pole was removed (area 10 and part of area 11, Walker) after ligation of the sagittal sinus. This failed to produce any hyperactivity either early or late (2 months) after the procedure. The similarity in activity before and after operation is shown in Fig. 3. The experiment involving ablation of area 14 described below is further evidence

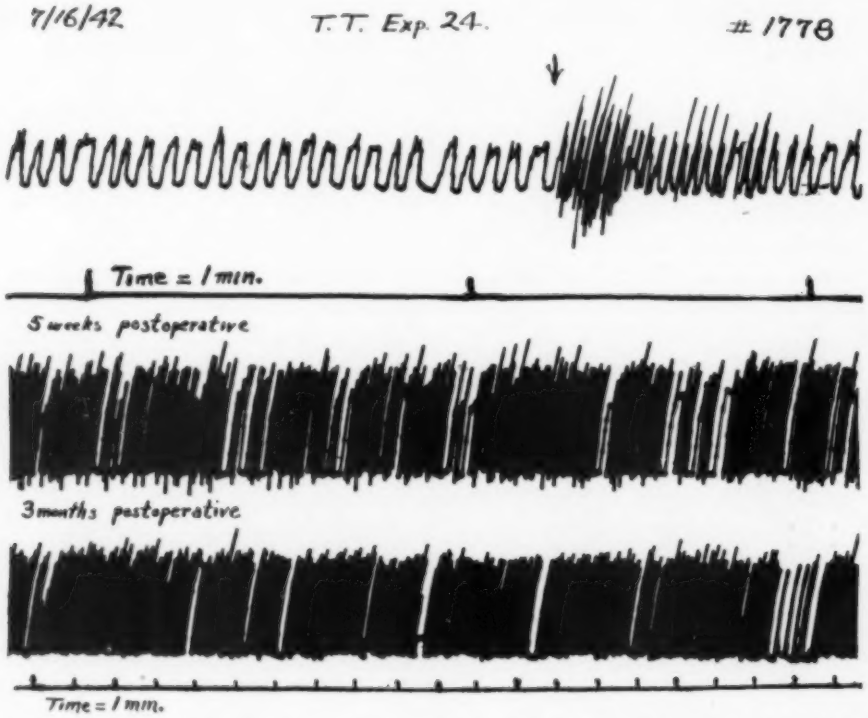

FIG. 2. Effect of bilateral area 13 lesion on activity. Upper record, made with a fast drum, shows speed and regularity of pacing. The record after the arrow shows accelerated running in response to presence of the experimenter. The two lower records demonstrate marked hyperactivity in the late postoperative period.

of the same weight. It is unlikely therefore that ligation of the sagittal sinus or the slight damage incidental to elevating the frontal pole would account for the hyperactivity.

Specificity of area 13 lesions. Preliminary experiments, too few to allow definite statement, suggest that portions of the orbital surface other than area 13 are without the same effect on hyperactivity. The ablation of the tip of the frontal lobe (area 10 and part of area 11, Walker) mentioned above accounts for the rostral portion of the orbital surface. In another monkey (T.T. No. 23) area 14, which occupies the posterior portion of the internal orbital gyrus (gyrus rectus), was ablated bilaterally. This animal was in

excellent condition for 5 days when it suffered a head trauma resulting in a subdural hematoma. During the first 5 days, careful observation revealed no similarity to the area 13 animals which all became definitely hyperactive within this period. At no time did he pace, exhibit diminished emotional behavior or ignore the observer. The animal's cage activity could not be distinquished from that of a normal macaque in an adjacent cage. The lateral region of the orbital surface has not been explored. From this limited expe-

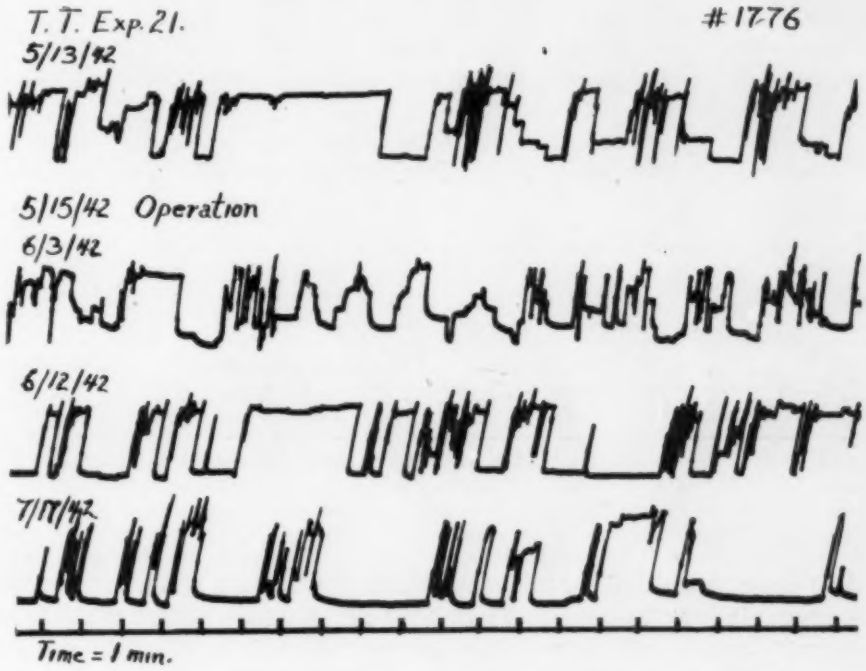

FIG. 3. Activity records showing normal activity after ablation of tip of frontal pole with ligation of the superior longitudinal sinus at its anterior end. Hyperactivity is absent both as an immediate and a late event after operation.

rience no suggestion is forthcoming that regions on the orbital surface other than area 13 are concerned to any large degree with hyperactivity.

Food intake, gastrointestinal motility and oxygen consumption. Somewhat the same ambiguity exists with respect to hyperphagia as a symptom of frontal lobe lesion as for hyperactivity; it has been found in the rat (20) but not in the monkey (11). Since afferent and efferent visceral connections of the orbital region have been demonstrated physiologically (1, 2) the food intake was measured before and after ablation of this region. In three area 13 animals the *ad libitum* food intake was determined for a standard diet made up of foods from the laboratory diet. Carrots, potatoes and bananas were fed in fixed amounts and peanuts and bread were allowed in unlimited quantities; the daily residues were sorted and weighed. Food intake was

expressed in Calories per kg. body weight. When averaged for a period of 5–10 days this value was found to be quite consistent from animal to animal. A comparison of pre- and postoperative food intake indicated a slight increase for two animals and a slight decrease for the third. (See Table 1.) In view of the metabolic cost of the hyperactive state, which is also reflected in a slight weight loss, no significance can be attached to these slight changes Ablation of area 13 certainly does not appear to cause true hyperphagia; the same result was obtained for prefrontal lobectomies by Kennard, Spencer and Fountain (11).

Since area 13, according to the stimulation experiments of Bailey and Sweet (2) is related to gastric tonus, and since the frontal lobe has been re-

Table 1. Food intake and body weight

Experiment No.		Preoperative	Postoperative
14	Av. C/day	306.00	366.00
	Body wt.	3.25	2.85
	C/kg.	94.00	128.00
16	Av. C/day	351.00	380.00
	Body wt.	3.25	3.00
	C/kg.	108.00	127.00
13	Av. C/day	310.00	291.00
	Body wt.	2.50	2.32
	C/kg.	124.00	125.00

lated to gastrointestinal motility by Watts and Fulton (24), carmine tests were conducted on 3 animals. There was no evidence of increased rate of transit of carmine, which is in agreement with the findings of Kennard, Spencer and Fountain (11) for other prefrontal lesions.

Resting rate of oxygen consumption was not increased in 2 area 13 animals. These observations will be reported in full by Dr. M. A. Kennard.

DISCUSSION

The hyperactivity produced by ablation of area 13 of Walker appears to be qualitatively identical with the behavior described as hyperactivity by previous workers. An important characteristic of the phenomenon which is not conveyed by the conventional terms "hyperactivity" or increased "random" or "spontaneous" activity is that the phenomenon is manifested chiefly in the sphere of locomotion. No hyperactivity of membral movements, of eye and head movements, of facial expression or in the "posturing" of the animals could be detected. On the contrary activity in other than the locomotory sphere appears to be reduced. Certainly the infinitely varied play of postures as well as emotional expression normal to the macaque is largely lost. Quiescence rather than agitation characterizes the cage be-

havior when not pacing. In short, the release of activity appears to be fairly specific and not a generalized increase of activity. The early view that the frontal lobes exert some *general* "inhibitory" effect on cortical or subcortical centers is therefore untenable.

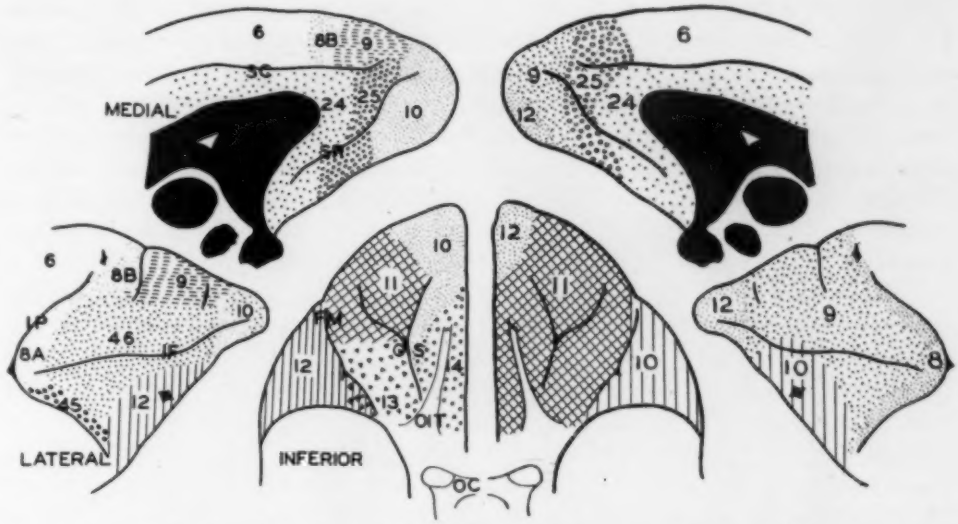

FIG. 4. A. E. Walker's cytoarchitectural map of the prefrontal lobe of *Macaca mulatta* (left) compared with Brodmann's map of *Cercopithecus* (right). The numerical designations in Walker's map are derived from Brodmann's designations of comparable areas in the human cereberal cortex and hence do not necessarily have anything in common with Brodmann and Vogt's designations for the monkey.

A feature of the hyperactivity induced by lesions of area 13 which indicates that this region is especially associated with the control of activity is the rapidity of its onset after operation. In all monkeys characteristic pacing was observed on the morning of the first postoperative day. Objective records of activity showing typical bouts of walking were obtained on the first day in the apparatus, which was 1 to 3 days after operation. They differ from later records in the length and frequency of pauses and in the rate of walking but the bouts of walking are of the characteristic methodical, stereotyped pattern. An early onset of hyperactivity seems to be unique to area 13 lesions. Kennard *et al.* (11), for example, state that "The hyperactivity is usually preceded by a period of hypoactivity immediately following the operation which may last a few days or weeks. During this time, the animals appear confused, lethargic, slow and difficult to arouse." Their animals for the most part had fairly large lesions of the prefrontal area. Richter and Hines (21) also noted that the onset of hyperactivity was delayed (*e.g.*, 20 days after bilateral lesion of area 9), when lesions were limited to the cortex of the frontal association areas, while the onset was more precipitous after striatal lesions.

Consistent with the rapidity of onset and development is the excessive degree of hyperactivity induced by this relatively small ablation. Within two weeks of operation the hyperactivity appears to approach that reported for large prefrontal lesions, though exact quantitative comparison is difficult. One of the criteria of maximal hyperactivity, continuous running for periods of an hour or more, is met by area 13 animals. The standard drum speed is too slow to allow exact calculation of the rate of running in all records; however, for one animal it is 20 round trips per min. in a cage 4 ft. long. A record made with a fast drum is shown in Fig. 2. While the hyperactivity of area 13 animals appears to exceed that of prefrontal lobe animals in the first weeks after operation, the phenomenon may be more persistent in the latter. Finally, as pointed out in the review of literature, large as well as small prefrontal lesions unaccountably fail on occasion to induce hyperactivity. It has occurred consistently in a short series of 4 area 13 animals.

There are then three indications that area 13 is an important area for the control of activity; hyperactivity produced by ablation of this area approaches in magnitude that from large prefrontal lesions; it sets on more quickly after operation; and it seems to be less variable in occurrence, degree and time of onset. The critical area appears to be area 13 and not the whole orbital surface. At least, two bordering areas, 14 and 10 plus a part of 11, have been extirpated as a primary procedure without producing any sign of hyperactivity of early onset. On the whole, the degree and rapidity of onset of hyperactivity induced by ablation of area 13 would seem sufficiently pronounced to preclude its being due to damage to other prefrontal areas.

The existence on the orbital surface of an unsuspected area related to activity raises the question whether it has been included in lesions designed to destroy all prefrontal tissue. Area 13 occupies the most posterior portion of the orbital gyrus (also called the posterior orbital gyrus, 2). It is not unlikely that in attempting a prefrontal lobectomy and especially a lesion of areas 9–12, area 13 may be included or excluded from the block by deflecting the ablating instrument either slightly anteriorly or posteriorly with relation to the frontal plane. Any variation from lesion to lesion at the orbital surface might contribute to the variability in degree of activity produced by large prefrontal lesions. Furthermore area 13 is so situated that it is likely to be spared by prefrontal ablations designed to preserve the striatum, and ablations designed to include the striatum are likely to include area 13 as well. The converse of the latter is also true. However, intense hyperactivity can result from a lesion (Fig. 5) which spares the caudate and damages only slightly the inferior border of the putamen. No cellular reactions suggestive of ischemia of the striatum were discovered. Mettler and Mettler (18) implicate the striatum in the production of a phenomenon termed "forced, cursive hyperkinesis," the relationship of which to the hyperactivity described above is not clear.

It has been argued (17) that hyperactivity from prefrontal lesions is of delayed onset and that from striatal lesions is of immediate onset

so that the thalamus rather than the cortex controls activity through the striatum. Since area 13 lesions which do not damage the caudate nucleus (Fig. 5) produce immediate hyperactivity this argument loses weight and leaves open the possibility of an area 13-striatal connection for the control of activity.

Summary and Conclusions

The posterior portion of the external orbital gyrus (posterior orbital gyrus), which Walker has recently differentiated as a new cytoarchitectural area (area 13) and which Bailey, Bremer and Sweet (1, 2) have demarcated physiologically from adjoining areas, has been ablated in a series of monkeys. This procedure produces in a marked degree many of the symptoms that have been described for prefrontal lobectomy by various workers under the term hyperactivity. The results of area 13 lesions are as follows:

1. Hyperactivity is manifested by long continued, methodical pacing or running of a regular, stereotyped character.

2. Hyperactivity from area 13 lesions is quantitatively great, is consistently obtained and is always manifested in some degree within the first or second postoperative day; whereas similar hyperactivity from other prefrontal areas is said to be delayed in onset (as long as 2–3 weeks) and does not invariably occur.

3. Ablation of neighboring regions by the same operative approach was without effect on activity.

4. Other motor activities are not marked by hyperactivity but rather suffer reduction. Random, spontaneous activities and posturings are reduced in variety and quantity, as is emotional expressivity. There are also

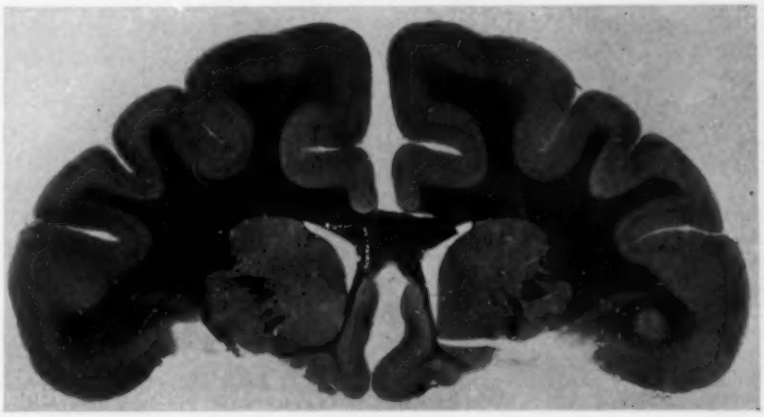

Fig. 5. Frontal section through the brain of a monkey made hyperactive by ablation of the posterior orbital gyrus of both hemispheres. The ablation extends to the inferior border of the striatum which is only slightly damaged. Expt. T.T. no. 13.

certain ill-defined behavior changes. All of these are most marked in the first postoperative week.

5. Hyperactivity is accompanied by a weight loss and only a slight increase in food intake.

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RESPONSES TO ELECTRICAL STIMULATION OF SINGLE SENSORY UNITS OF SKIN

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INTRODUCTION

IN SPITE of numerous and elaborate studies on skin sensibility, the cutaneous receptors have yet to be adequately investigated by one of the useful methods of neurophysiological analysis, that of direct electrical stimulation by single and repeated shocks. Although this is not the normal means of activating such receptors, the handicap here is no greater than where this method is applied to the study of nerve trunks or nuclei of the central nervous system. In fact the use of artificial or unaccustomed methods of approach has the advantage of minimizing the prejudices that arise from customary experience. Certain elementary questions still remain undecided after extensive study of sensory receptors. The receptors which serve the different modalities of sensation are still in dispute, and the earlier inference that pain endings were more superficially located in the skin than touch endings has recently been challenged (14, 15). The very character of the excitation process in the receptor is unknown. Whether a persistent excitation of the receptor induces repetitivie excitation of its nerve fiber as a constant current does, or whether the sense organ itself responds repetitively, remain equally problematical.

To approach some of these problems a method was sought for effectively stimulating single sensory receptors without mechanically deforming the skin. A small electric spark was employed, and the device of Fig. 1 was used for preliminary exploration of sensory receptors in human subjects. The sensory experiences reported were taken as data. These data, combined with what is known about nerve responses to various forms of sense organ stimulation in animal experiments, allow certain inferences to be drawn as to the physiological behavior of the sense organs concerned. The observations hereafter reported may call for further checks on animals under similar conditions of stimulation.

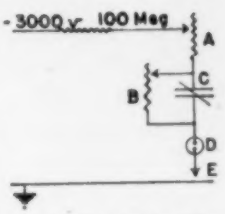

FIG. 1. Diagram of simple apparatus for stimulation of single sensory endings without mechanical contact. See text.

In Fig. 1, direct current at 3000 V. potential from a filter circuit charges the whole stimulating structure through a protective resistance. *A* and *B* are carbon line variable resistors of several hundred megohms each, *C* is a variable condenser consisting of concentric test-tubes

* Assisted by a grant from the Rockefeller Foundation for Research in Neurophysiology.

wrapped in tinfoil. *D* is a small neon tube in the handle of the needle holder that carries the stimulating needle *E*. When the latter approximates the skin to about 0.5 mm., the lower plate of *C* discharges through the needle and air gap to ground potential with a flash signal in *D*. If resistances *A* and *B* are too high to permit a steady flow of current across the gap, the discharge is a single brief shock, and the needle can be raised again before the condenser is recharged through *B*. At lower settings of the resistances a repetitive discharge is permitted at a frequency determined by the values of capacity and resistance selected.

The three types of endings revealed by the Von Frey hair technique and its variants are readily identified by electrical stimulation of single endings; those mediating ordinary touch or light pressure, those for touch associated with hair shafts, and those inducing the sensation to be identified as prick, which becomes pricking pain on a stronger stimulation. Occasional spots sensitive to cold have been encountered which also responded to electrical stimulation, but none for warmth; these two have not been specifically explored for.

Responses to Single Stimuli

On regions of skin where sense organs are relatively far apart, prick and touch can be readily differentiated by spark stimulation. One suitable location is the dorsum of the web between the fingers. Here prick has a much lower threshold than touch to electrical stimulation of single endings This is also true for the back of the hand and arm generally, although the opposite is true of the skin covering the balls of the fingers. The difference is in part assignable to differences in depth of endings below the surface, for if the skin of the ball of the finger is sandpapered sufficiently the order is reversed, the prick threshold becoming equal to or lower than that for touch as is found elsewhere. The extreme sensitivity of the fingertips to touch may be due to the greater number of receptors there rather than to the great sensitivity of individual receptors, in the case of mechanical as well as electrical stimulation, for as will be indicated below, the activities of adjacent touch receptors are strongly summated subjectively.

A single stimulus applied to a single prick spot at the threshold for subjective identification is not painful, but elicits a tactile experience usually accompanied by a faint aura of itch. The latter is especially noticeable if the stimulus is repeated immediately. This tactile sensation from a single receptor is so faint as to be recognized only when attention is closely paid to it. It has a long latency, and is not associated with a feeling of pressure. As the stimulus is increased, a stinging or pricking sensation is induced, with a rapidly shortening latency and an increasing after-effect of prick which often fades to itch. A single strong stimulus has an effect which is sharp, stinging and persistent. On the other hand a single threshold stimulus applied to a touch ending is experienced as a slight tap. As the stimulus is increased, the tap feels heavier, but does not persist. The latency of the

sensory experience is far shorter than that for threshold prick. Strong single stimuli become unpleasant, and as they do so pain may be felt; but exploration of territory adjoining the receptor with a stimulus of constant strength usually reveals a sharp focus of this painful sensation at a nearby prick spot, with a steady increase of prick sensation in passing from the previously identified touch spot to its neighboring prick spot. The prick resulting from strong electrical stimulation of an isolated touch spot is therefore in the larger part or perhaps wholly due to spread of current to adjacent lower-threshold prick endings. Over the forearm few pure touch areas can be found although one encounters with a weak stimulus numerous foci of pure prick without accompanying sensations of touch. The reason for these relations is that the electrical threshold for prick is in general considerably lower than for touch. Touch can be recognized with stronger stimuli as a feeling of abrupt contact or tapping against a background of pricking pain.

The thresholds referred to are those eliciting sensory experience, and are not demonstrably the thresholds for initial activation of a nerve fiber by its receptor. Since the nerve fiber's impulse is all-or-none, if one impulse in one nerve fiber resulted in a definite sensation, a slight increase above its threshold should at least double the sensory effect experienced, and this should be recognizable as a step-like increase in sensation. No such unit augmentation is recognizable, even when experienced from touch spots where the sensation at apparent threshold is rather definite. Therefore it is inferred that even threshold *sensation* experienced from the stimulation of *one receptor* involves the repetitive response of the nerve fiber leading from that receptor, and as the number of nerve fiber responses increases with increase of the stimulus, the sensation increases by fractions too small to be recognized as other than a smooth gradation.

The third group of receptors to be considered here, touch organs at the bases of hair shafts, are more similar in their responses to touch organs generally than to prick endings, but are not identical to these. They will be considered more fully below. The sensation induced by a single stimulus to a hair shaft receptor is indistinguishable from that induced by bending a hair mechanically. It is not experienced as a tap or light blow, as is the stimulus to other touch receptors.

PERSISTENT AND REPETITIVE EXCITATION OF SINGLE SKIN RECEPTORS

Technique: For repetitive stimulation the simple apparatus noted above was not subject to precise control, since the value of the stimulus varied with the distance across the gap, and with repetitive discharges the first stimulus was greater than the succeeding. There is also a lower limit to the energy that can be delivered across such an air gap, and this limiting value proved to be above threshold for many sense endings. The apparatus of Fig. 2 was therefore devised for more accurate and flexible manipulation. A constant high voltage is applied through a high resistance vacuum tube and switch H to maintain ionization of the air gap G. Pulses from the same source are applied to the variable condenser F and thence to G, by means of an electronic tripping device at A. The intermittent shocks discharged across G stimulate the skin at L, during an interval that can be limited by the short-circuit K, and at a strength regulated by F and also by the shunt circuit at J. Be-

side maintaining a constant condition at the gap, the direct ionizing circuit through *H* also acts with the circuit through *F* as a voltage-doubling device, permitting the use of a small condenser and brief time constant of the arc discharge.

The operation of the device is rendered audible in ear phones at *I*, one of which is connected in each of the two parallel circuits, through the body and through the shunt, a click being delivered to one ear when a stimulus passes through the skin, otherwise recording only in the other ear. The stimulus cycle can be photographed on the oscillograph *O*, whose sweep is timed with *K*. A tap key *N* signals on the oscillograph the approximate latency of the subjective sensation. Alternately *H* can be thrown to deliver only constant current to the circuit beyond the gap *G*, or at open position a constant (or high frequency) arc discharge occurs across *G*. The technical details of this apparatus are given in the appendix below, together with certain physical considerations involved in the operation of an arc at low current values.

With the body grounded through the recording circuit, stimuli are applied through a fine wire in a needle holder, the contact of the wire itself being too light to be felt; or a spark will jump to the skin if the wire is held under a hand lens near the skin. Alternately

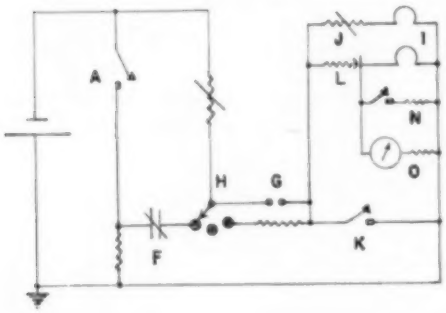

FIG. 2. Schematic diagram of apparatus for delivering repetitive electrical stimuli to a single sensory ending. Description in text, and further details of construction and operation in Fig. 6.

we have employed thin mica disks 1 cm. in diameter, with a fine central perforation over which a 2 mm. disk of foil was laid, and the whole compressed so that the foil extrudes through the perforation to make contact with the underlying skin. Such disks contain 1 mg. of mica and 2 mg. of foil, can be dropped on the skin without being felt, and are fixed in place by smearing the edges with vaseline. Contamination of the dry skin with vaseline seems to offer no impediment to the current, and may facilitiate electrical contact, as it does on metal surfaces generally. Moistening the skin surface with salt solution or distilled water on the contrary increases the effective threshold for stimulation, probably by diffusing the current above the highly resistant cuticle.

If a repetitively discharging needle is drawn slowly cross the dry skin, under observation through a binocular, apparent prick loci, that is points of strongest sensation, are most frequently encountered in the depressions between polygonal areas of mechanically stiffer cuticle. It is probable therefore that one of the chief factors determining the thresholds of single sense organs of the skin for electrical stimulation, and to some extent for mechanical stimulation, is the structure of the skin itself and its mosaic of electrically and mechanically resistant areas.

Responses to repetitive stimuli. There has been considerable disagreement in the literature concerning the quality of sensation with relation to intensity of stimulation. The question is usually presented in the form: Do all receptors mediate pain when sufficiently stimulated, or is pain a single modality mediated by its specific sense organ? In a previous paper (9) we concluded the latter, with the reservation that a single stimulus to a few nerve fibers passing to potential pain endings caused only tactile sensation. It is perhaps academic to argue whether an incipiently painful sensation

shall be called painful in anticipation, or tactile in retrospect, since it is naively obvious that a sensation is painful only if it is painful *enough*. There is however distinct qualitative difference between even threshold sensations from stimulation of regions that on stronger stimulation elicit respectively stinging or pricking pain and superficial touch or light pressure.

Stimulation of what will hereafter be designated touch receptors, with weak single shocks, gives the impression of a light blow, and this sensation of abruptly applied pressure is only intensified on moderate increase of intensity. It is simply repetitive at low frequencies, with slight temporal summation. At higher frequencies the sensation becomes smooth pressure. At higher intensities it becomes unpleasant, especially at low frequencies, and is usually contaminated with pricking pain due to spread of current to adjacent prick endings. Usually where touch is at a maximum for a given intensity of stimulus, prick is at a minimum and vice versa.

In contrast, repetitive stimulation of what will be designated hereafter as prick receptors, even stimulated at a threshold where a single stimulus is not painful, rapidly summates to frank pain, without any of the feeling that a blow has been struck. In fact a distinctive characteristic of weak repetitive stimulation is the gradual onset of this sensation, doubtless related to the long latency to weak stimuli. It is impossible to obtain from prick receptors alone the vibratory character of the sensation so readily elicited from touch receptors.

The tactile organs at the bases of hair shafts lie in some respects between these two. The latter organs, (which will be designated hereafter as hair-receptors, in spite of the fact that other receptors often occur here,) are difficult to stimulate through the skin surface, probably because they lie deep at the root of the hair-shaft. They are readily stimulated by a high voltage shock led to the proximal *1* to *2* mm. of the hair above the skin. By this circumstance they are reliably differentiated from other touch organs under electrical stimulation. Single weak stimuli give a sensation identical to that caused by slight bending of the hair shaft. Repetitive stimulation results in summation to a steady sensation at considerably lower frequencies than that characteristic of touch organs, but they show less summation than do prick endings. They do not give the sensation of a tap on the skin surface, as do touch organs; they rather give the feeling of something rough drawn across the hair. Strong stimulation via hair shafts always results in pain, and from some hairs only pain can be recognized, the pain then masking the tactile sensation if a hair-receptor is indeed present. On the other hand, a few hair shafts have only ordinary skin touch in their vicinity.

The fact that one finds such a varying relation between the thresholds for prick and for tactile sense associated with hair shafts indicates that the two sensations must be mediated by different organs, lying at varying distances from the hair shaft. Whether the hair tactile organs themselves give pain on strong stimulation cannot be finally decided under these circumstances. We have not been able to find any hairs that failed to cause pain

when pulled from their moorings, but the threshold for pain even to gross pulling of single hairs is very variable. The sudden jerk with which hairs are most expeditiously removed is sometimes not painful, but always leaves an after-effect of itch, (see below.) A few hair endings have been found where the threshold for prick was at least ten times that for touch.

In connection with hair-organs, the following observation is perhaps significant. If a single hair shaft is poked with a fine instrument, the hair must be very considerably bent before any sensation is induced. If now a hirsute area is disturbed so lightly, even with a breath of air, that any single hair is bent less than its threshold for recognition requires, the sensation is unmistakably more intense. Aside from the obvious demonstration of spatial summation, this observation indicates that the threshold for subjective sensation to sense organ activation is materially higher than the threshold for response of the nerve fiber itself; in other words, more than one nerve impulse from one sense organ is required for a sensory effect. This is analogous to the repeated finding in direct stimulation of nerves leading to central nervous system responses as recorded electrically; more than one impulse over one nerve fiber is often required to fire a central synapse.

Distributions of sensory endings as tested by electrical stimulation. A stimulus pattern was chosen by trial which gave the most clear-cut differences in sensation from prick, touch, and hair-shaft endings respectively. This proved to be a continuously repetitive sequence at about 5 per second, varied in intensity for different types of ending necessarily, but held constant otherwise. The needle was raised from the skin between trials, which consisted each of about 1 second contact. The subject was the present writer, normal so far as indicated by clinical skin tests. He had the cues of sound in the ear phones, vision of the needle through a *X2* hand lens, and the oscillographic trace of the stimulus record when significant. Areas were explored at three locations along the lower arm, on the back of the hand, and on the nail bed, balls of fingers, and palmar surfaces of phalanges.

Prick receptors. Stimuli which individually caused slight prick at the most sensitive points showed summation at 5 per sec. At this strength the spots were sharply localized, and moving the needle as little as 1/10 mm. was sufficient to abolish all sensation. With increase of strength these points expanded, and other points were found, the intensity of sensation decreasing with distance from such focal points. With stimuli strong enough to induce light pressure sensations, prick could be elicited from almost any area, but with striking differences in intensity, varying between burning pain and slight prick. Drawing the needle across the skin at uniform rate gave the feeling of irregular jumpy movement, with occasional sharp jabs as if the point had suddenly caught and penetrated the skin. Distances from one prick focus to another varied, from 2 to 10 mm. on the arm and hand, (Fig. 3) but due to poor subjective localization below threshold for touch, it is difficult to decide whether two adjacent spots are referred to the same locus or not. Consequently it cannot be judged whether one nerve fiber supplies

more than one prick focus, although a unit field almost certainly includes an area around each focus.

Such a distribution of excitability for prick might be due to several causes; to varying depths of sensory endings below the skin; to variations in skin resistance; or to variations in intrinsic excitability between end branches of one axone. That one factor is either variation of skin resistance or of depth below surface is indicated by the fact noted previously that many "high" points for prick lie in minute grooves between more stiff areas of skin. Also, if the skin is shaved closely, or the epidermis removed with sandpaper, the electrical threshold is materially lowered. In such an area, a scratch by a grain of sand through the epidermis, even too slight to cause irritation, has a greatly lowered threshold to prick. That is, one can produce a "high" spot by removing the cuticle locally, and normal variations in skin resistance will therefore be a factor in such localization.

Touch receptors. Skin shaved a few days previously will contain stubs of hair shafts long enough to manipulate but too short to interfere with exploration for touch. The spatial unit for the latter sense appears to be a relatively uniform area rather than a high point grading off to relative insensitivity. These areas are a centimeter or more in diameter toward the elbow, grading to a few millimeters on the back of the hand, and one or two millimeters on the ball of the finger (Fig. 3). Usually, but not always, each area will have one point of definitely greater sensitivity to a given strength of stimulus. This can be located within less than half a millimeter, with no obvious relation to irregularity of the skin over it (Fig. 3 E). Subjectively, the sensation at that point is stronger, and at least while watching the needle, stimulation of other parts of the area seem to be referred to this point. Above threshold, stimulation at any point within an area feels the same, except at the high point, that is, there is not the progressive gradation from peaks to valleys found associated with prick foci. The margins of adjacent areas may overlap, or there may be narrow spaces between them without touch sensation unless the stimulus is very strong (Fig. 3 B). On hirsute surfaces many of the hair shafts are situated at the peripheries of touch areas, some areas lying entirely between hairs. An exception was noted on the back of the hand, and the hair endings here gave a sensation when stimulated that could not be distinguished from ordinary touch, which in fact was probably also stimulated. The margins of these areas are located by drawing the needle slowly across the skin, marking points where the sensation shifts abruptly in subjective localization. It appears obvious that these are the endings involved in compass-point tests, of which the above maneuver is a modification, though it cannot be said that each area is served by one fiber.

In Fig. 3 tracings are presented of groups of touch areas. It will be noted that the high points for prick show no correlation with touch areas, and there seem to be more prick spots than touch areas, at least over the forearm and hand. On the balls of the fingers this is not obviously the case, but here the

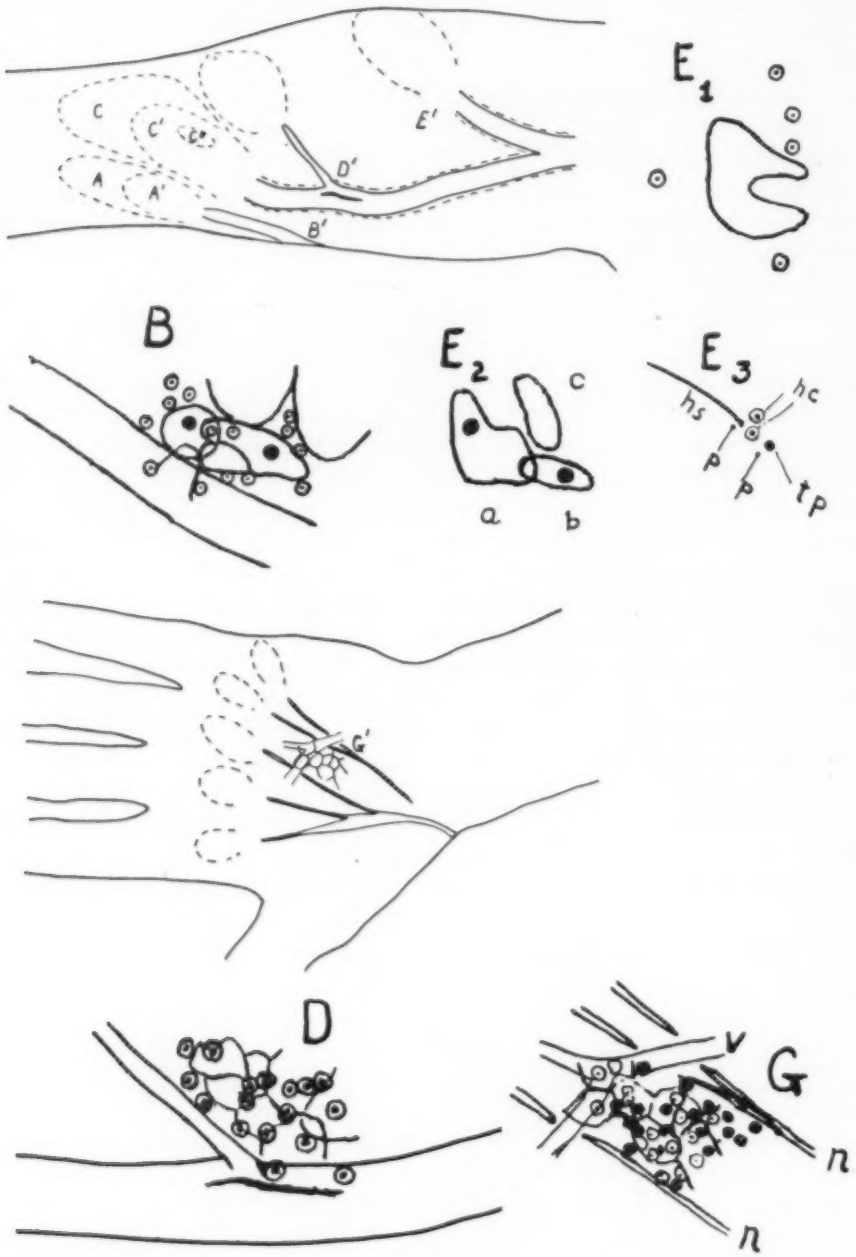

FIG. 3. Diagram of dorsal surface of right forearm, and of the right hand, ½ size, to illustrate effects of electrical stimulation of nerves and of sense organs in and below the skin. All stimuli involved a 2300 V. source, employing various capacities at *F* in Fig. 2. Pain was reached at the most sensitive skin points with the variable capacity of 0.0001 Mf set at about ½ of this maximum value. Employing 0.001 Mf, nerve trunks and branches could be stimulated over wide paths as outlined in full lines on forearm outline, and a sensation of

density of distribution of both appears to be greater. The needle, discharging repetitively and drawn slowly across the ball of the finger, *seems* to change its position in a saltatory manner about every two millimeters or less, and these apparent shifts of position presumably indicate the margins between the smaller adjacent touch areas in this region. Prick here requires a much stronger stimulus than does touch, and its threshold is so greatly reduced by removing the cuticle, that the number of separate prick spots is impossible to estimate. On the region just proximal to the ball of a finger, prick again has the lower electrical threshold, and the distribution of prick points is not greatly different from that over the arm.

Foci of touch and prick located by electrical stimulation were checked by pricking with a sharp needle. These electrical high spots for prick coincided exactly with the points where minimum pressure was required for mechanical prick. When a touch area had a similar high focus, this was also more sensitive to light touch than its immediate surroundings. Otherwise there seems to be more variation in touch threshold when the mechanical

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touch was referred to the areas enclosed in dash lines. At 0.0011 Mf the paths were broader, indicated by dash lines parallel to the full lines. At 0.0002 Mf the only region where a nerve trunk could be stimulated was along the heavy line below D', where the nerve presumably lay closest to the surface. The nerves in hand outline were located with 0.0005 Mf stimulating capacity. The widening of a pathway presumably indicates a shallower depth of the nerve. Crossing above the nerve branches at G is a vein v through which the nerves could not be stimulated.

The areas of reference seemed to contract as the nerve stimulus was made weaker; A and C correspond to the strongest stimuli, A' and C' to stimuli near the edges of the paths, and C'' to the threshold response from along the line below D'.

B, unit touch areas outlined with a 0.0001 Mf stimulus; drawn actual size. ⊙ indicates hair shaft, ● is the high spot for touch in its area. Three of these areas overlap with a fourth, while between the right hand one of these and the area above, a space is vacant for touch. In this space three hair shafts could be investigated for hair-sense uncomplicated by ordinary touch. Located at B' on outline of arm.

D, touch areas near a nerve trunk, at D' on outline, 0.0003 Mf stimulus employed. 0.001 Mf gave no referred responses here except along nerve paths as outlined. Hair shafts indicated by circles.

E, three details near location E'. E_1 a single touch area containing no hair shafts. E_2, three adjacent touch areas of decidedly different affect with the same stimulus. *a* gave a very strong abrupt sensation, with the spot marked by a black circle even more sensitive, to which any stimulus in this area seemed to be referred. The sensation from *b* was less pronounced, but it also had a focal point of reference. From *c* the sensation was weak and poorly localized. E_3, an area about a single isolated hair shaft, *pp* are pain foci, *hs* the hair shaft, *hc* two separate hair endings, one immediately at its base, one further away and at one side. *tp* a touch area high spot, where a pain ending adjacent to it was stimulated at about threshold for touch. Over the pain ending itself the threshold for pain was much lower, and the location of the pain spots could be confirmed by mechanical stimulation with a sharp needle. The proximity of the other pain spot to its nearest hair ending made it impossible to obtain hair touch without pain, but this could be induced at the more distant hair ending.

G, area on back of hand between nerve branches, located at G' on outline of hand. Touch areas are outlined, circles are hair shafts, dots are high points for pain. Stimulus 0.0001 Mf for pain spots, 0.0003 Mf for touch. There seems to be no correlation between the distributions of touch, pain, and hair shafts.

needle is employed, than with electrical stimulation. Across the ball of the finger a moving mechanical contact gives an apparently smoothly changing localization, as contrasted to the step-like progress with electrical stimulation. This may be assigned to the gradual deformation of the skin ahead of the mechanical stimulus.

Hair receptors. The distribution of sense receptors associated with hair shafts appears to be one to each hair, or if there are more than one they must lie very close about the follicle. The sensation cannot be localized subjectively with sufficient precision to judge whether one fiber distributes to more than one hair, but this can certainly not be excluded. The point on the skin which, stimulated electrically, gives a sensation identical with moving a hair, lies usually one fourth to one half millimeter from the hair shaft's point of emergence, in the direction of the hair's slant under the surface, and when an occasional case is found where the sensation is not contaminated by either prick or light touch, this point can be located with the needle very precisely. Not all hair follicles however have a characteristic modality. A few have no sensory points in their immediate vicinity; some give only prick, even when a spark is sent along the shaft itself; and some have only ordinary touch near them. For a few, no specific sense organ could be found electrically, but bending still produced a sensation, perhaps from a sense organ too deep in the skin to be reached by other than currents strong enough to confuse the issue.

Functional correlation with histological structure. Such rather distinctive distributions of the excitable areas for different senses obviously suggests the possibility of correlating sensation with known histological structure, but it must be recognized that to the many pitfalls which have complicated such attempts in the past, the method of electrical stimulation adds its appropriate quota. Beside the considerations already mentioned of variations in skin resistance and depth of endings below the surface, the question arises what is being stimulated, nerve fibers, their fine end-ramifications, or specialized endings, or the non-nervous enveloping tissues of sense organs.

The specialized structure of at least some sense organs, consisting of non-nervous capsules surrounding the modified ending of a nerve fiber, obviously suggests the function of transforming one kind of energy—that of the external stimulus, mechanical deformation or temperature change for instance which corresponds to the receptor's modality—to another kind, whatever that may be, which is peculiarly suitable for setting up impulses in nerve fibers. The fact that even the briefest stimulus, a sudden prick or an electric shock, can set up a persisting sensation, which must involve a continuing train of discrete nerve impulses in a nerve fiber, strongly suggests that at some site in the sense organ a persisting chemical or physicochemical effect has been induced, which is not all-or-none, and which serves as the intermediate stimulus to the nerve. In fact since the "excitable" part of the sense organ is presumably its nervous part, we might differentiate this

process further, to the effect that a first stage might consist of the production of an exciting agent by non-nervous tissue, the second stage, of the excitation of a specialized nerve ending, which then activated its axone. For purposes of analysis then the peripheral sensory process can be considered physiologically at least as a three-stage one: first, the transformation of energy from a special to a general form, which makes the sense organ specific for a certain type of stimulus; second, the utilization of that energy in the setting up of a persisting state of excitation; and, third, the transformation of a persistent excitation into a repetitive train of impulses. It would be convenient, even if not experimentally justifiable, to identify as the loci of these stages, the non-nervous tissue, the expanded sensory nerve ending, and the nerve fiber respectively. The nerve fiber at least is in character, for it customarily responds with a repetitive train of impulses to a persistent stimulus, whether this be a constant current, a local injury, or a chemical agent.

Certain features of the sensory response to electrical shocks indicate that it is not the nerve fiber itself which is directly stimulated. The long latency of threshold prick is probably a function of a slowly established peripheral process, not characteristic of nerve fibers activated by brief shocks, and the narrow precision with which certain prick "high" spots and all hair endings can be located argues for the activation of a discrete and limited structure, not a random segment of a nerve plexus such as terminal fibers are known to pass through. In plotting touch areas, one occasionally finds, with strong stimuli, that the area to which the sensation is referred is a distant one; that is, a small superficial nerve branch has been stimulated directly. (Fig. 3). The fact that with weaker stimuli touch areas can be accurately circumscribed with the stimulating needle without reference of any sensation to an external area is convincing by contrast. To stimulate prick *fibers* in such superficial nerve branches requires a stimulus so exorbitant as not to be comparable to the shock required for prick *receptors* under the needle, which are in contrast the most easily stimulated of all skin endings. It must be concluded that specialized endings, especially for prick, are more easily stimulated by electric shocks than the fibers leading from them. If as inferred above, one impulse in one fiber fails to arouse any sensation at all, then the ability to detect sense organs by such brief shocks as we have used itself indicates that specialized endings rather than fibers are being stimulated, for these shocks at any reasonable intensity could only set up one impulse each in any fiber they reached.

Finally, the distribution of endings correlates fairly well with the terminal nerve ending distributions of the skin to which Woollard and collaborators (17, 18) assign the functions of touch and pain. However, the data presented herein are not sufficient to conclude whether the touch endings lie more superficially than prick endings. Their failure to find any specialized endings for the fibers which they assign to pain may compromise

the argument presented above that specialized endings rather than ordinary nerve fibers are stimulated, except that they do mention varicosities, etc. along the terminal branches. It may be suggested that such minor modifications may serve as the seat of the physiological specialization which the results of electrical stimulation seem to demand.

Certain Theoretical Considerations; Form of Sensory Message in Terms of Neurone Responses

Whatever the validity of the foregoing inferences, it is obvious that the immediate result of a single stimulus to a skin receptor, however brief, is a single or repetitive response in a fiber leading from it. Differences in sensation due to differences in strength of single stimuli must involve differences in number and frequency of such impulses. In fact, any sensory affect, at the peripheral nerve level, must be mediated by means of specific and all-or-none impulses in the nerve fibers from sense organs. Further, the physiology of the central phenomena evoked must be dealt with in terms of such impulses impinging on central synapses. It will be convenient at this point to evaluate the types of activity induced by our procedures in terms of nerve impulses.

The impulses in a peripheral nerve fiber are variable in two ways only: first, in number, and second, in frequency. The lower limit of response of one nerve fiber to stimulation of its sense organ is one nerve impulse, and this probably fails to reach the higher centers unaided. Various experimental evidence from work on animals (1, 12, 8, 19) indicates what the further stimulation of a sense organ above its nerve threshold means in terms of nerve impulses in its fiber. A stronger stimulus excites a sense organ more intensely, and its excitation will persist for a longer time. The intensity of excitation appears in terms of frequency of discharge of impulses in the nerve fiber, its persistence in terms of numbers of discharges. To a first approximation the frequency of nerve discharge at any instant may be taken as a measure of the intensity of the sense organ's excitation obtaining at that moment, or vice versa.

For the present purpose, and without experimental evidence on the point, sense organ excitation may be assumed to be inherently non-rhythmic and not all-or-none.* The time-intensity course of such excitation may be

* No one in fact knows how a sense organ responds; our picture of the process is derived by inference from the response of the fiber activated by it. The possible exception is the visual sense cell, the potential of which was recorded by Hartline (8) in the eye of limulus, separate from the potentials of nerve elements associated with the sense organs. If this directly recorded potential is a measure of the state of excitation of the receptor cell, in the same sense that a nerve impulse seems to be a measure of its fiber's response, then the nerve fiber from a photo-receptor responds repetitively to a persisting and non-periodic excitation in its sense organ.

The other view is presented by Matthews (12) in connection with a study of muscle stretch endings. He infers that the sense organ itself responds repetitively to constant stimulation, *i.e.*, physical stretch distorting the excitable membrane, "since there is no

then considered to have the form of the smoothed summation of the all-or-none responses of its nerve fiber, as indicated in Fig. 4*AB*. The effect at any synaptic region may again be evaluated as a smoothed summation of those same impulses, unless they are spaced too far apart to be summated smoothly, or at all.* How exactly the curve of intensity of subjective sensation will duplicate the curve of excitation of the sense organ, will depend on how the pattern of impulses in nerve fibers is modified as it passes central synapses, for instance, by the resistance of the synapse to single impulses.

With a qualification to be noted below, the repetitive response of a nerve fiber following a single brief stimulus applied to a sense organ for touch or prick does not induce a repetitive *sensation*, that is a vibratory sensation. This means that the lowest frequency at which the sense organ can cause the nerve fiber to repeat, is still too high for resolution by higher centers as repetitive sensation, and indicates an upper limit on the rate at which repetitive stimulation can be recognized as such. Employing repetitive

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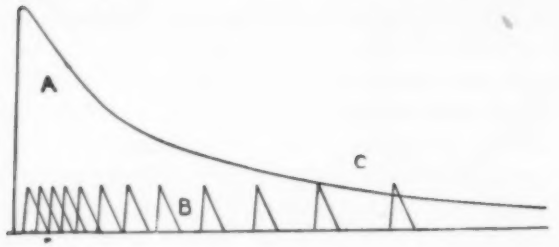
reason to suppose that the end-organ differs essentially from any other excitable structure," . . . and he assigns to it a refractory phase similar to that of nerve, as a property of its polarisable membrane. He obtained no records from the sense organ itself.

* This summation picture is suitable for the present representative purposes, whether summation at synapses takes place actually by building up a persisting excitatory state in the neurone, or whether it involves the activation of auxilliary pathways which reinforce a first impulse by delayed impulses, or whether both events occur in complicated centers. Concerning the actual nature of a presumed central excitatory state, that is, a persisting state of excitation at a synapse or group of synapses, support for both of the above views may be found in recent literature. For instance, Lorente de Nó (11) has shown that in the oculomotor system a volley of impulses may take two paths, one direct to a synapse causing an immediate post-synaptic volley, and one by a roundabout or "reverbering" circuit over which impulses arrive later at the same synapse, causing a scattered after-discharge. On the other hand, Bronk (6) has shown that cells of the single-synapse station in the stellate ganglion are themselves capable of after-discharge following adequate repetitive stimulation, indicating a persistence of excitation at each synapse. Whatever the actual process occurring at a synapse or at a group of them, its effect on post-synaptic fibers can be represented as a smoothed curve, and this curve may be interpreted according to the predilections of the reader as a persisting excitatory state, or as the mean of a series of discrete excitations.

A reconciliation of these two views may be derived from some work of Lloyd (9a) on efferent nuclei activated via the pyramidal pathways of the cord. Certain large cells whose axones could be discretely recorded from, responded only after considerable summation of impulses from adjacent units, and then responded with a rhythm different from that of the initial stimulus to the pyramids. Lloyd remarks, "It is probable that the highly asynchronous activity of the small units in this region . . . constitutes a slowly changing, statistically smooth over-all excitation to the large solitary cell units, and that these latter in turn respond at intervals determined in part by their own properties." This leaves undefined the exact locus of initiation of the all-or-none type of response, which for the time being is just as well.

The significant point for the present discussion is the fact that impulses meet with something like inertia or impedance at synapses, such that an increasing effect is produced by successive impulses, and the energy used in overcoming this inertia may be later delivered as an after-discharge. In this the synapse is similar to a sense organ, for instance a prick ending, where an instantaneous stimulus may cause an effect only after a latency, but the effect persists long after the stimulus is removed. The general result is a damping of the excitation sequence as in Fig. 4, B-D-G.



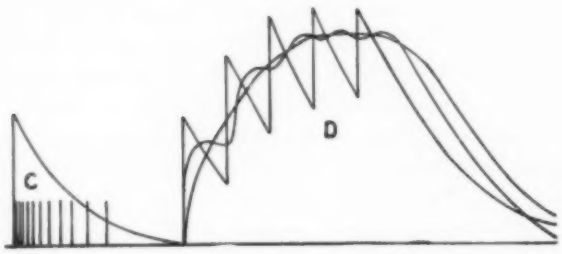


FIG. 4

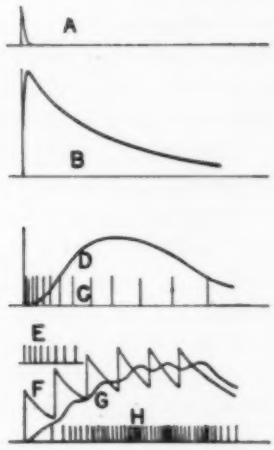


FIG. 5

FIG. 4. Diagram to illustrate the relation between sense ending and nerve, or between afferent fiber and the synapse to which it leads, on the assumption that the excitation in sense organ and at the synapse is persistent, in contrast to the discontinuous character of nerve fiber impulses. *A*, the triangles of constant amplitude represent a train of impulses in one fiber, decrementing in frequency. The overlying curve represents the course of the excitation in a sensory ending set up by a very brief stimulus and persisting at a decrementing intensity after the stimulus is removed. The decrementing *intensity* of the excitation of the sensory ending corresponds to the decrementing frequency of nerve impulses of constant intensity. It is supposed for purposes of representation that summation of the nerve impulses at a central synapse will reverse this transformation, resulting again in a persisting excited state of decrementing intensity.

C, the initial figure is a duplication of *A* on a slower time scale, the triangles represented as vertical lines. The saw-tooth curve represents a succession of such responses to a repetitive stimulation of the sensory ending, assuming that the frequency of stimulation is such that recovery is not complete between stimuli. The undulating and smooth curves superimposed on this represent increasing degrees of smoothing of the summation of impulses at a center, depending upon its character or upon the number of synapses traversed, etc. A repetitive excitation may thus be smoothed to give a continuous sensation, which may persist after stimulation ceases as the after-discharge of the summated excitation.

FIG. 5. Diagrammatic representation of the pattern of excitation at successive levels of the nervous system, based on the assumption made in Fig. 4. *A*, the potential-time form of a brief electric shock applied to a sense organ. *B*, the intensity-time course of the sense organ's excitation. *C*, the repetitive response of the afferent fiber to stimulation of its sense organ, assuming that the frequency of that response is at any moment proportional to the intensity of *B*. *D*, the smoothed curve of summation of *C* at a synapse, assuming that several impulses are required to excite it, involving a delayed rise of excitation. *E*, a train of impulses corresponding to *C*, condensed to the time of the first peak of the saw-tooth curve *F*, representing nerve fiber and sense organ excitation respectively. The whole curve *F* represents the summation of a succession of curves *B* upon repetitive excitation of a sense organ. *G* represents the summation of successive responses such as *D* at a synapse. *H* is then the series of impulses in the nerve fiber beyond the synapse, responding to the varying intensity of *G* with a periodically varying frequency. The delayed start of this train of impulses corresponds to a long latency of sensory effect, and the varying frequency of *H* corresponds to a vibratory character for the sensation. When the changes of frequency

direct nerve stimuli to skin nerves in human subjects, touch fuses into a smooth non-repetitive sensation at not over 60 per second (9). Pain fuses at 30 per sec., even with strong nerve stimulation. With repetitive stimulation of prick *endings*, each individual stimulus of which obviously sets up a train of impulses as compared to the single impulse of direct nerve stimulation, so that summation readily takes place, fusion of the sensation occurs at a lower frequency than with stimulation of the nerve itself. The condition can be diagrammed as in Fig. 5 *H*, where repetition as a sensation is a function of the periodic fluctuation in frequency of nerve fiber discharge. This is analogous to the conversion in radio receivers of a frequency-modulated carrier wave to an amplitude-modulated audio wave. This is essentially the picture obtained by Bartley (4), for visual flicker fusion as observed in electrical records from the optic cortex.

In fact, by pushing this analogy a little farther than experimental evidence at present warrants, the passage of a sensory message from the periphery to the center may be pictured as an alternation of amplitude modulation of excitability in cells or junctions, and frequency modulation in nerve fibers connecting them. The character of the neural message is then expressed in terms of frequency modulation of all-or-none, that is maximal-amplitude, nerve impulses in the fiber pathways, and this will be converted to amplitude or intensity modulation at each cell station.

This scheme, whether literally correct or not, may be employed as a convenient graphic representation of the results of experimental procedures. It suggests the application of patterns of stimuli to single sense organs or definable groups of them, to test how the central nervous system deals with such patterns, as revealed in the pattern of sensation resulting. The apparatus at present available delivers only the simplest patterns, regularly repetitive stimuli of controllable number, frequency and intensity, adequate for activation of one ending at a time. Its limitations can be obviated at the expense of only a slight mechanical complexity, but it is suitable in present form for studying some of the attributes of sensory response.

Relation of Quality of Sensation to Pattern of Sense Organ Stimulation

At any given strength or frequency, the variation in number of stimuli, that is in duration of stimulation period, has little or no effect on the quality of sensation after the first few shocks. The sensation simply persists, with minor changes in intensity of an adaptive character. Prick builds up to a maximum and then decreases slightly. Touch feels like an initial tap, with continuing steady pressure if the frequency is high. Hair touch stimulation produces a constant sensation with little or no decreases after the abrupt

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of *H* become too rapid, or too slight to be followed by higher centers, the sensation will be a continuous one; that is the summation will have been completely smoothed as indicated in Fig. 4 *D*.

start, in contrast with the slow rise of the sensation of prick. The adaptation to steady electrical stimulation is definitely less for either touch or prick than is that to steady mechanical pressure, which suggests that the chief adaptation is in the initial sense organ process, while electric stimulation may act beyond this, on a later stage of the excitatory sequence.

More pronounced changes in quality are correlated with changes of frequency and intensity of a repetitive stimulus. Well above threshold, a single shock to either a touch or a hair ending induces a brief but definitely rough feeling, as if it consisted of a short period of rapidly repetitive stimulation. This is not duplicated by mechanical stimulation. With high-frequency shocks this roughness disappears. It seems possible that this sensation reflects a real repetitive discharge in the nerve fiber, each impulse in the brief train which follows a single shock registering discretely in the central nervous sytem, until the rate of stimulation exceeds that which the centers can follow. Failure of this phenomenon with mechanical stimulation could then be accounted for by the circumstance that skin deformation would usually stimulate more than one ending, even if only of one axone, and the impulses reaching the centers would not be so regularly timed. No such impression is ever induced by any stimulation of prick endings.

The most striking change of quality is derived from the latter. A single stimulus little above threshold induces only a slight prick; if strengthened, a sharper prick is followed by definite itch. If repeated, at 5 or less per sec., the prick seems to be constant but the after-effect of itch builds up with successive stimuli. Various combinations of frequency and strength were therefore tried, with the aim of inducing only a feeling of itch from a single ending, without any initial pricking pain. This result is readily obtained with the combination of weak shocks and high frequency; shocks so weak that a single one is not painful, and several are required to summate for a definite itch, and frequencies anywhere from 10 per sec. up to a steady arc discharge. The higher the frequency, the less the strength must be for the purest sensation of itch, except that a steady current seems to be less effective than a fairly high frequency. After a few seconds of effective stimulation the itch persists for many seconds, and just below intensity required for frank pain, it is slight exaggeration to describe it as intolerable. Stronger stimulation causing pain is less disagreeable, and the itch is promptly allayed by scratching.

Attempt to induce tickle were less successful. Weak and rapid stimulation of hair endings came nearest to it, touch endings failed completely. In a few instances where a prick spot near a hair shaft happened to give the right combination, the tickle seemed to be enhanced by what would otherwise have been a slight itch. This together with common experience might lead one to infer that tickle requires stimulation of more than one ending, perhaps of more than one kind of ending, and could be compounded of touch and itch in the right proportions,—and especially if experienced against a more re-

ceptive emotional background than that which motivates its dispassionate analysis.

An occasional hair ending which could be stimulated to give hair-touch alone, without either pressure or prick endings being activated, showed a peculiar partial adaptation, in that, when stimulated slightly above its threshold at high frequency, the sensation changed in quality during the course of the constant-strength stimulation. The initial effect was identical with that caused by bending a hair, but this sensation faded into a mild tickle, which persisted as long as the stimulation, without significant after-effect. This is in marked contrast to similar stimulation of prick spots, which caused an increasing itch with a long-persisting after-effect. To describe the one as due to partial adaptation, the other as due to summation, implies that these two processes are simple opposites, and this may be an over-simplification, for the hair-endings seem to show adaptation in the sense of responding more weakly after an initial period, but also summation in the sense that a repetitive stimulus results in a non-repetitive sensation. Pending actual observations of nerve impulses in animals after similar stimulations, these terms should be taken only in a descriptive sense.

Discussion

An adequate understanding of the physiological basis of sensation would require a knowledge of the activity as well as of the structure of each unit along a path between the sense organ and the cortex, to match against a report of the sensation involved. Most of the descriptions of the activity of parts of the central nervous system have been derived from synchronous stimulation of many parallel fiber pathways, on the presumption that the responses of similar elements synchronously activated would be equivalent to the activity of one element of pathway stimulated through a single fiber. This is certainly only a first approximation to the truth, even allowing that one *element of pathway* through the central nervous system probably involves a number of parallel neurones. Recent anatomical work indicates that even in the sensory periphery the functional unit of pathway is not a single fiber and its end-arborisations. Weddell (15, 16) reports that groups of Meissner's corpuscles not over 0.15 mm. apart and innervated each by a separate fiber, constitute a touch spot; and that each hair may be innervated by two separate fibers. Further the areas innervated by the end terminals of fibers presumably mediating pain in the skin overlap one with another. In the cornea Tower (13) found that stimulation of any area within the field of one fiber caused that fiber to respond, and one fiber and its branches extended over as much as one quarter to one half the cat's cornea, plus neighboring regions of the sclera, as indicated by recorded responses of single fibers to mechanical stimulation of the cornea. Here again overlap occurred between fiber fields. The pathway for sensation is apparently multiple even from the periphery.

On the other hand, the interpretation put upon investigations of sensory response has been largely colored by subjective reports of localization of the stimulus, and by the naive inference that the finding of a sensory "spot" that could be identified uniquely, indicated that this spot might be innervated by a single fiber, connected uniquely to a corresponding spot in the cortex. This idea has broken down completely as applied to the one sensory area where acuity of spatial discrimination is most refined, that is in the retina. Not only is the overlap of innervation in the neurones of the layers of the retina itself enough to preclude it, (8) but further overlap takes place at the geniculate level (11a).

The work cited on innervation of the skin suggests that here also the subjective recognition of discrete loci of stimulation is not to be accounted for by simple one-to-one connections between sense organs and cortical neurones. While points of extreme sensitivity are found in the skin, for both touch and prick, by electrical stimulation as well as mechanical, it does not seem probable that these points are the *unique* loci of the characteristic end organs capable of giving the same sign. Around each prick spot, however sharply localized, prick can be induced by a slight increase in current strength, and around each touch spot is a touch area capable of similar activation. The differences in strength of shock over positions having different thresholds are not sufficient to account for stimulation of one central spot by diffusion of current; the unit such as it is, is an area covered practically continuously by sensory endings, and apparently by endings of more than one fiber.

The apparent uniqueness of sensory foci must then be a function of the relative thresholds of such endings, due either to differences of intrinsic sensitivity or to differences of accessibility to the stimulus, etc. Woollard *et al.* (18) emphasize in fact that even with mechanical stimuli, localization must be considered to be three-dimensional. The notorious difficulty in identifying histologically the unique endings in excised areas in which sensory spots had been previously located may be accounted for in part by the multiplicity of such endings, the apparent uniqueness being a function of accessibility to stimulation.

To the extent that such considerations are valid, the distinctions that are tacitly made between mechanisms for general skin sense and for special sense may be dispensed with, and with them may be abandoned the notion that any modality is primitive, in the sense that it is served by a simpler or less elaborate neural mechanism. Rather one may look for a general scheme in accordance with which the nervous system receives and handles afferent messages, and differentiates between the intensity, quality, and spatial extent of an excitation. The function of hearing a sound of frequency above what any one nerve fiber can transmit raises the same problem as that of recognising a mechanical vibration of frequency higher than any one skin touch organ seems able to follow, and the explanation of visual acuity, when arrived at, should apply to the problem of skin localization. The findings of

Derbyshire and Davis (7) that the auditory nerve fibers respond alternately when the frequency of stimulation exceeds the capacity of any one fiber, and of Bartley (2) that the cortex responds at half frequency when the frequency of light flashes to the retina exceeds the ability of the cortex to respond to each, indicate one expedient employed by the nervous system to overcome the limitations of its individual elements with respect to frequency of response. The scheme hypothecated by Lorente de Nó (10) designated as "partially shifted overlapping" proposes another by which it might deal with the limitations of spatial distribution. It may be anticipated that recognition of quality of sensation, exemplified crudely in peripheral terms as difference in local sign, will involve more than a spatial localization in the brain of projection areas corresponding to sense modalities, although the neural mechanisms actually appropriate to this function have not yet been suggested. The notion that pain is a function of the thalamic centers and discrimination that of the cortex is presumably only the same sort of too simple rationalization to account for quality of sensation, as is the assumption of point-to-point correspondence between brain and periphery for spatial discrimination.

Even though electrical stimulation of one ending of one fiber may be possible, it does not follow that the central nervous system transmits and interprets the impulses induced in a correspondingly simple manner. The chief virtue of such experimental restriction of activity to one peripheral element may be the demonstration that such stimulation is insufficient to enable the central nervous system to obtain an adequate estimate of external conditions. It may be inferred that the central nervous system is designed not primarily to detect discretely the activity of each ending, however precisely it may incidentally accomplish this, but rather to integrate the activities of many; and even peripheral sense organ activity will not be satisfactorily analysed, at least in terms of subjective sensory response, without a further understanding of the central mechanisms that react to it, as well as a knowledge of what each sense organ does to its immediate nerve fiber.

Summary

Single sensory spots in the skin of human subjects can be conveniently stimulated by high voltage, low current spark discharges, without mechanical deformation of the skin.

The distributions of sensitivity over various regions, for touch and prick, show characteristic patterns. "High" spots of extreme sensitivity to electric stimulation are surrounded by areas of lower sensitivity. An area, varying in size in different regions, from less than two mm. to more than fifteen mm., appears as a unit in the sense that any stimulus within it is referred to the same locus.

Prick has a much lower threshold than touch, except on the balls of the fingers, where touch threshold is lower.

Tactile endings associated with hair shafts can be differentiated from

other tactile endings by the different sensory effects from electrical stimulation of suitable pattern.

Itch without accompanying prick can be elicited by low intensity, high frequency stimulation of prick endings. Itch also follows as an after-effect of slowly repeated stimuli, each of which causes an initial sharp prick. The same sensory spot can give either touch, prick, itch or sharp pain, all below threshold for ordinary touch endings.

Certain theoretical inferences are offered as to the action of sensory endings, as indicated by the responses obtained by electrical stimulation.

APPENDIX

The apparatus of Fig. 6 consists of the following divisions; a high voltage rectifier and conventional filter, the bleeder circuit only of which is figured; a thyration stimulator consisting of trip tube and two stages of amplification, delivering repetitive shocks through the condenser *F*, whose controls are *A B C* and *E*; a constant current tube controlled at *D* and a switch *H*, by which either constant current or repetitive pulses can be selected; an air

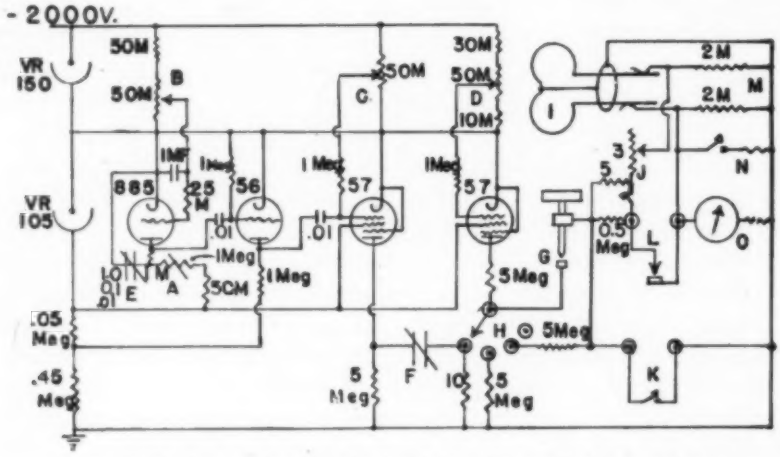

FIG. 6. Technical details of stimulator. See text of appendix.

gap *G* and an alternate 5 MΩ fixed resistance; and the stimulating and recording network *I* to *O*. With *H* at the left hand point, the intermittent and steady currents from the two pentodes serve as a voltage doubling device at the spark gap. At the second point the output is grounded making this the "off" position. At the third the constant current to the stimulating circuit is shunted around the air gap, and at the fourth, the only path for this current is across the gap, resulting in a steady or very high frequency arc discharge. The stimulating needle at *L* is a #40 wire 5 cm. long whose mechanical contact with the skin cannot be felt, and in operation the spark will pass to the skin without touching it. The point of employing the air gap at *G* as a high resistance in series with the skin is to minimize variations of skin resistance at the contact. The resistance of dry skin even under contact with a clean needle may be so high that a shunt at *J* of 1 or 2 MΩ will render the current through *L* inaudible in its ear phone, and ineffective as a stimulus, and wide variations in skin resistance occur within fraction of a millimeter, as indicated by sound in the phone when so shunted. The spark gap thus serves as an effective current controlling device in a circuit of variable resistance; the wider the gap is set, the less does skin resistance variation affect the current.

The air gap activated by brief impulses alone proved to be not only variable in operation, but limited in the quantity of current which would pass it in one pulse. This lower

limit is imposed by the circumstance that a certain initial quantity of current is utilized in ionizing the air in the gap, after which the arc resistance is abruptly lowered. A condenser small enough to suit the present purposes loses so large a fraction of its current, even at high initial voltage, in ionizing the gap that the residual voltage after ionization is not sufficient to cause an arc; or if the initial quantity of current is increased, the subsequent arc discharge through the lowered resistance may be above threshold for a sense ending. If there is a critical value that would be theoretically suitable, its range is too narrow to be practical. A value of capacity and voltage is therefore employed which is safely above this critical and unstable level, and part of the current may be wasted in the shunt circuit, which acts as a fine adjustment.

To overcome the inconstancy of operation of this spark, the constant current delivered through the pentode and switch *H* is held just at discharge level, indicated by a faint buzz in the phone, and this current is far below threshold for skin sense organs. The variable condenser *F* is also charged by this steady current in one direction, through the grounded resistance to its left hand terminal; it is discharged and charged in the opposite direction by the impulse, adding the two voltages at the moment of discharge, and permitting the use of a very small condenser with a short time constant in the transient branch of the circuit.

Two thousand ohms are shunted across each ear phone, to permit operation if the plug is not inserted. The tap key *N* employed to signal latencies of sensation, operates by grounding the oscillograph input, the amplitude of this signal depending on the value of resistance in series with *N*. The short-circuit switch *K* is on a rotating interruptor which also controls the oscillograph sweep, and by adjusting the duration of the opening of *K*, one or more of a sequence of repetitive shocks are applied to the skin and recorded on the oscillograph at each revolution of the interruptor. The apparatus is constructed to deliver currents making the stimulating needle at *L* cathodal, but the anode seems to be equally effective and construction might be simplified by reversing the polarity of the stimulus generator. The body has sufficient capacity so that a ground connection is not necessary, except to carry the impulses to the recording apparatus. The spark gap,works more constantly if the anode is needle-pointed. The condenser at *F*, of the radio transmitting type, has a maximum capacity of about 0.0001 Mf, and fixed condensers can be inserted in parallel for stronger stimulation. The design and construction of the apparatus are due to Mr. R. G. Loeffel.

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ACTION POTENTIAL AND ENZYME ACTIVITY IN THE ELECTRIC ORGAN OF *ELECTROPHORUS ELECTRICUS*. II. PHOSPHOCREATINE AS ENERGY SOURCE OF THE ACTION POTENTIAL

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INTRODUCTION

RECENT investigations suggest that the release of acetylcholine is intrinsically connected with the nerve action potential and may be responsible for the electrical changes occurring at the neuronal surface during nerve activity (7, 19–24). The new concept of the physiological role of acetylcholine is based mainly on the following facts: (i) on the high concentration of choline esterase in nerves, which shows that significant amounts of acetylcholine can be metabolized within milliseconds; (ii) on the localization of choline esterase at the neuronal surface; and (iii) on the close parallelism between the concentration of choline esterase and the maximum voltage of the action potential which may be demonstrated on electric organs.

The changes at the neuronal surface during activity and their rapid reversal cannot conceivably be effected without energy loss, since they must involve processes which are—from the thermodynamic point of view—irreversible, and can only be reversed by the free energy of chemical reactions. If the release of acetylcholine and its subsequent breakdown is responsible for the alterations of the nerve membrane during the transmission of the nerve impulse, chemical reactions must supply the energy for the resynthesis of acetylcholine.

The most readily available source of energy for endergonic life processes is the energy of phosphate bonds. In muscle, the breakdown of adenosinetriphosphoric acid appears to be the primary energy source for the contraction, possibly directly connected with the mechanical changes (4, 18). The adenosinediphosphate formed is rephosphorylated by the breakdown of phosphocreatine, a phosphate shift which occurs without loss of energy. Phosphocreatine thus acts as a "storehouse" or "accumulator" for energy rich phosphate bonds (14). Ever since phosphocreatine was found in nerve it was assumed that it may have a function there similar to its function in muscle and may yield the energy for the action potential (6, 8, 11). This possibility

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was tested by Gerard and his associates (9, 10). In ordinary nerves, however, only general estimates are possible since both electrical and chemical changes are too small for such studies. The large electric organ of *Electrophorus electricus* offers a suitable material for comparing electrical and chemical changes of the action potential since both are in the range of possible measurement. Experiments carried out on these organs and described in this paper show that the energy mechanism of the action potential is fundamentally identical with that used in muscular contraction.

METHODS

a. *Phosphates.* The method of Fiske and Subbarow as applied by Lohmann (16) was used. The tissue frozen in liquid nitrogen was ground in iced trichloracetic acid (5 per cent) and filtered. For the determination of the true inorganic phosphate an aliquot portion of the filtrate was taken and within less than three minutes added to a solution of ammonium magnesium citrate prepared according to Mathison (17). Another fraction of the filtrate was directly analyzed, indicating the true inorganic phosphate and phosphocreatine phosphate. A third fraction was hydrolized in *N* HCl at 100°C. for 15 min. to obtain the phosphate of adenosinetriphosphate. For the determination of the total phosphate one fraction was ashed with 2 *N* sulfuric and nitric acids. A photoelectric colorimeter was used.

b. *Lactic acid.* The method described by Barker and Summerson (2) was used in which a pink color was developed by p-hydroxydiphenyl. This determination also requires a photoelectric colorimeter.

c. *Creatine.* The creatine was transformed into creatinine and determined by Folin's open flask method.

RESULTS

A. *Distribution of phosphorylated substances and creatine.* The presence of phosphocreatine in the electric organ was first described by Kisch (13), who suspected that this compound might be connected with the activity of the organ. Baldwin and Needham studied a series of enzymatic reactions in extracts of the electric organ, particularly those connected with phosphorylated compounds. They showed that some of the main steps known to occur in intermediate muscle metabolism also occur in those extracts (1).

The observations mentioned above were carried out on the electric organ of *Torpedo*. In the electric organ of *Electrophorus electricus*, the concentration of phosphocreatine is as high as in striated muscle and in some cases even considerably higher (see Table 1). In specimen No. 1 the values of phosphocreatine are on the average about 2 mg. P_2O_5 per gm., which is a concentration about 50 per cent higher than in frog's muscle. This is particularly remarkable in an organ with 92 per cent water content. The concentration of adenosinetriphosphate (ATP), although lower than in muscle, is still high: 0.2–0.4 mg. P_2O_5 of ATP phosphate. In contrast to the variations of choline esterase the distribution of phosphate and phosphorylated substances does not decrease from the head to the caudal end of the main organ.

Creatine is also evenly distributed and the concentration is higher than in muscle and in the electric organ of *Torpedo*. About 500–600 mg. per 100 gm. of electric organ were found in medium sized specimens as compared with 300–400 mg. in striated muscle. In the Bundles of Sachs, which releases much less energy per gm. than the main organ, both phosphocreatine and creatine

are markedly lower than in the main organ. Only 0.3–0.4 mg. P_2O_5 were found as phosphocreatine per gm. of electric organ. The creatine content was 250–300 mg. per 100 gm. No ATP was found, although it is probable that the compound is present and that the amounts are too small for determination by indirect estimation in the presence of the other phosphates.

Table 1. Concentration and distribution of phosphocreatine (PhCr) and adenosinetriphosphate (ATP) in the electric organ of Electrophorus electricus. Four samples of the second specimen were taken from the Bundles of Sachs. (B.o.S.)

Specimen No.	Distance from the anterior end cm.	P_2O_5 directly determ. mg./gm.	PhCr in mg. P_2O_5 per gm.	ATP in mg. P_2O_5 per gm.	Total P_2O_5 mg./gm.
1	5	3.17 2.98	2.23 2.08	0.19 0.20	3.43 3.03
	8	3.15	1.76	0.19	3.60
	25	3.30 3.07	2.41 2.14	0.16 1.10	3.57 3.57
	30	2.94	1.90	0.25	3.32
2	6	2.61 2.40	— 1.40	0.39 —	3.16 3.11
	8	2.35 2.72	1.39 1.27	0.32 0.39	— —
	27	2.35	1.50	—	2.76
	30	2.54 2.54	1.27 1.21	0.39 0.31	— —
	58 B.o.S.	0.59 0.59	0.43 0.37	0 0	0.74 0.84
	63 B.o.S.	0.57 0.54	0.34 0.37	— 0	— —

B. *Breakdown of phosphocreatine as result of discharge.* A few preliminary experiments were carried out to see whether phosphocreatine is split as a result of the electric discharge. The fish was taken out of water and laid in an insulating trough in which two electrodes 10 cm. apart made contact, each over an area of some $cm._2$, with the skin covering the main electric organs. These were joined by a resistance of 30 to 100 ohms in order that the discharge should release energy by an electric current. A piece of electric organ from the part between the electrodes was rapidly cut out and thrown into liquid nitrogen. After varying periods of stimulation by light prodding alternating with periods of rest, other pieces were cut out and similarly frozen.

The number of discharges during each period of stimulation was roughly counted by watching a cathode ray oscillograph connected to the electrodes.

Three such observations are recorded in Table 3. The experiments show that the developments of the action potential are associated with breakdown of phosphocreatine. Moreover, at the time when the fish shows marked fatigue, phosphocreatine has fallen to a low level. In Expt. no. 3 for instance the organ appeared during the second period of stimulation to be very fatigued. The phosphocreatine level was already low at that period. When, after 3 min. rest, stimulation started again, the organ appeared nearly completely fatigued and during twelve minutes of prodding only a few discharges of low voltage occurred. During this period the phosphocreatine did not change measurably: 0.39 and 0.35 mg. P_2O_5 of phosphocreatine phosphate respectively were found.

The rate of recovery appears to be slow. In experiment 1 for instance there is no measurable resynthesis of phosphocreatine after 5 min. rest on the table and 20 min. recovery in water. Only after 20 more min. in water a synthesis had occurred. The initial value was in this case rather low, so that the synthesis is still rather small, although the amount is nearly as high as before the stimulation period.

C. *Correlation between electric energy and chemical energy released by breakdown of phosphocreatine.* By the experiments just described the breakdown of phosphocreatine was linked to the discharge. A quantitative comparison was next undertaken between the energy released by the breakdown of phosphocreatine and the electric energy delivered to the external circuit in a large number of discharges. The number of discharges was counted by means of an electric "scaling circuit" by which every sixteenth discharge was indicated. The oscillograph was used with a camera to obtain photographic records of the voltage. A number of exposures were made at different times during the period of stimulation. The electric power released externally is V^2/R, where R is the resistance of the *external* circuit and V is the voltage between its terminals. The voltage varies continuously during the impulse but is shown at each instant by the oscillographic trace. The instantaneous power could thus be computed at a number of different instants during the discharge, and from this computation a graph of the power vs. the time could be drawn. Since the energy is the time integral of the power, its value could be found by a graphical integration of the power-time graph.

The cross section of the organs could not be measured directly, since the fish were not killed, but the girth of each fish was measured and the cross-sectional area was estimated by comparison with sections of other electric eels on the assumption that the area is proportional to the square of the girth. The fish were all of similar size, so that the relative areas were probably determined in this way with fair precision. From the average energy of one impulse and the estimated volume, the energy per impulse per gm. of electric tissue was computed, the specific gravity being taken as unity.

It was found that the electric energy was regularly near its maximum when the resistance was 100 ohms. Consequently this resistance was used in all experiments after the first few. In every experiment, immediately before and after the counted series of discharges, a piece of electric tissue was quickly cut out and thrown into liquid nitrogen and the phosphocreatine was later determined. The energy released per gm. of electric tissue by the break-

Table 2. Concentration of creatine in different sections of the electric organ of Electrophorus electricus

Specimen No.	1				2					3
Distance from the anterior end in cm.	8	10	30	45	10		40	60 B.o.S.		near ant. end.
mg. tissue used	850	548	522	512	567	621	490	521	543	669
Creatine mg. per gm.	*490*	*548*	*526*	*649*	*530*	*490*	*550*	*300*	*260*	*603*

Table 3. Breakdown of phosphocreatine (PhCr) during the discharge of the electric organ of Electrophorus electricus

I a before, b after stimul. (13 min., about 2000 discharges), c 5 min. later (fish kept in air) d after 20 min. in water, e after 20 more min. in water. II a before, b after stimul. (5 min., about 500 disch.) c 11 min. rest, then 10 min. stim. (about 800 disch.) d 7 min. rest then about 200 disch.; marked fatigue. III a before, b after stimul. (12 min., about 2000 disch.) c 3 min. rest, then 4 min. stimul. (about 700 disch.); marked fatigue, d. 3 min. rest, then 12 min. stimul.; nearly completely fatigued.

Exp. No.		P_2O_5 directly determ. mg./gm.	PhCr in mg. P_2O_5 per gm.	ATP in mg. P_2O_5 per gm.	Total P_2O_5 mg./gm.
I	a	1.89	*0.81*	0.23	2.15
	b	1.93	*0.35*	0.25	—
	b	2.04	*0.49*	0.17	—
	c	2.01	*0.32*	—	2.19
	d	1.94	*0.27*	0.25	—
	e	2.04	*0.85*	0.17	2.23
II	a	2.95	*2.07*	0.12	3.23
	b	3.00	*1.57*	0.22	3.26
	c	2.72	*0.86*	0.13	2.86
	d	2.85	*0.96*	0.19	3.10
III	a	2.23	*1.23*	0.12	2.35
	b	2.03	*0.49*	0.10	2.15
	c	2.06	*0.39*	0.10	2.16
	d	1.96	*0.35*	0.06	2.07

down of phosphocreatine was divided by the number of discharges to give the energy per gm. per impulse for comparison with the electric energy reckoned on the same basis.

In the first experiment, the number of discharges was 320. The resulting breakdown of phosphocreatine was small for quantitative measurement, and in the next two experiments the number was increased to 800. The energy of the phosphocreatine breakdown per gm. and impulse in these three experiments had the values 34.4, 36.9, 85.0 g.Cal. $\times 10^{-6}$ as compared with values 9.9, 14.3, 7.0 for the external electric energy. Even with 800 discharges the breakdown of phosphocreatine was smaller than was desirable. Since no fatigue was evident during 800 discharges the number was increased to 1600 in all the following experiments. This number seemed about optimum, as fatigue, when it was detectable at all, was evident only toward the end of the

Table 4. Breakdown of phosphocreatine (PhCr) in the electric organ of Electrophorus electricus during the discharge. Number of discharges: 1600. a. before, b. after period of stimulation. c. of exp. No. 6 was taken after 65 min. recovery in water. Figures separated by a line indicate values obtained by using different pieces, otherwise control determinations by using extracts of the same piece.

Exp. No.:		1		2		3		4		5		6			7	
		a	b	a	b	a	b	a	b	a	b	a	b	c	a	b
P_2O_5 directly determined mg./gm.		2.09	1.88	1.64	1.77	1.82	1.80	1.92	1.99	2.23	2.13	2.24	2.08	2.11	1.96	1.90
		1.93	1.91	1.93	1.76	1.78	1.81	1.95	1.86	2.24	2.25	2.07	2.07	2.12	1.95	1.98
			1.83								2.12					1.98
PhCr in mg. P_2O_5 per gm.	single	1.10	0.55	0.84	0.52	0.88	0.64	1.28	1.02	1.69	1.18	1.42	0.75	1.03	0.55	0.16
		1.14	0.41	0.84	0.47	0.89	0.68	1.28	1.01	1.69	1.17	1.41	0.75	1.01	0.79	0.19
		0.98	0.46	1.12	0.57	0.82	0.83	1.33	0.96	1.66	1.18	1.34	0.67	1.10	0.56	0.42
		1.01	0.52	1.12	0.50	0.81	0.87	1.24	0.86	1.69	1.08	1.33	0.62	1.10	0.65	0.40
			0.57							1.65	1.11					0.43
										1.66						0.47
	average	1.07	0.50	0.98	0.515	0.85	0.755	1.28	0.96	1.67	1.16	1.375	0.70	1.06	0.64	0.345
	split	*0.57*		*0.465*		*0.095*		*0.32*		*0.51*		*0.675*			*0.295*	

Table 5. Breakdown of phosphocreatine (PhCr) in the electric organ of Electrophorus electricus during the discharge. Number of discharges: 1600. a. before, b. after discharges.

Expt. No.	Time of excision	P_2O_5 directly determ. mg./gm.	PhCr in mg. P_2O_5		Expt. No.	Time of excision	P_2O_5 directly determ. mg./gm.	PhCr in mg. P_2O_5	
			per gm.	split				per gm.	split
8	a	2.38	1.52	*0.47*	12	a	1.98	1.36	*0.42*
	b	1.89	1.05			b	1.92	0.94	
9	a	1.85	1.15	*0.18*	13	a	2.00	1.345	*0.225*
	b	2.10	0.97			b	2.27	1.12	
10	a	1.89	1.235	*0.35*	14	a	2.01	1.30	*0.375*
	b	1.99	0.885			b	2.05	0.925	
11	a	1.76	1.07	*0.28*	15	a	1.59	0.97	*0.30*
	b	1.60	0.79			b	1.65	0.67	

series and was even then usually slight. The amount of phosphocreatine split, on the other hand, was sufficiently large, yet the phosphocreatine did not fall to too low a concentration.

It cannot be expected that all specimens will be in the same condition. There is a short period—usually about one min.—after they are removed from the water and before the first piece of tissue is cut. During that period

Table 6. External energy released by a section of the electric organ of Electrophorus electricus compared with the energy released by phosphocreatine breakdown in the same section during the same period. Length of section: 10 cm. The external resistance was 100 Ω. Number of discharges: 1600.

Expt. No.	Length cm.	Measured section		Electrical energy externally released		PhCr Split in whole section in mg. P_2O_5	P energy in whole section Cals.	PhCr split in mg. P_2O_5 per gm.	P energy per gm. & impulse Cal. $\times 10^{-6}$
		Girth cm.	estim. volume cc.	in whole section Cals.	Per gm. & impulse Cal. $\times 10^{-6}$				
1	185	36.6	400	9.2	*14.5*	228.0	32.0	0.57	*50.2*
2	179	36.0	380	5.9	*9.7*	176.5	24.9	0.465	*41.0*
3	182	36.5	390	5.6	*9.0*	37.8	5.3	0.097	*8.5*
4	185	39.0	440	5.6	*7.9*	141.0	19.9	0.32	*28.3*
5	161	34.5	350	5.3	*9.6*	178.5	25.1	0.51	*44.8*
6	167	38.5	430	5.4	*7.8*	290.0	41.0	0.67	*59.0*
7	172	35.5	370	5.0	*8.5*	109.0	15.4	0.295	*25.9*
8	171	37.5	410	4.7	*7.1*	192.5	27.0	0.47	*41.4*
9	182	36.7	500	6.7	*10.5*	72.0	10.2	0.18	*15.9*
10	183	35	360	4.5	*7.8*	126.0	17.8	0.35	*31.0*
11	182.5	38	420	4.5	*6.7*	118.0	15.7	0.28	*24.7*
12	171	37.5	410	5.3	*8.5*	172.0	24.2	0.42	*36.3*
13	161	37.5	410	2.9	*4.4*	92.0	13.5	0.225	*19.8*
14	190	38	420	3.7	*5.5*	157.5	22.2	0.375	*33.0*
15	181.5	36.5	390	3.4	*5.4*	117.0	16.4	0.30	*26.4*
Average:				5.5	*8.2*	147.2	20.7	0.369	*32.4*

some of them may give many discharges, others only a few. Variations of the initial concentration of phosphocreatine have therefore to be expected independently of the individual variations which may occur in the initial value in optimal conditions.

The results of phosphocreatine determination of 15 experiments carried out with 1600 impulses are summarized in Tables 4 and 5. The variations are in reasonable limits and the deviations from the average value not greater than those usually found for the breakdown of phosphocreatine for a given number of twitches of an excised frog's muscle. Only in Expt. no. 3 an exceptionally low value was obtained which may be due, partly at least, to the relatively low initial value. In the first seven experiments (Table 4) each piece was divided in 2 or even 3 parts while frozen and with each part two

control determinations were made of the true inorganic phosphate. Each value obtained was thus controlled several times. Since the two parts of the same piece generally showed a fairly good agreement, in the later experiments (No. 8–15, Table 5) the pieces were not divided, but still two controls were always made from each piece. The figures given in Table 5 are mean

Table 7. Lactic acid (L.A.) formation in the electric organ compared with the breakdown of phosphocreatine and the electrical energy released externally by the action potential. Number of discharges: 1600. External resistance 100 Ω. a) before b) immediately after stimulation c) during recovery.

Expt. No.	mg. tissue used	L.A. mg./gm.	L.A. formed mg./gm.	Energy released per gm. & impulse Cal. $\times 10^{-6}$			
				by L.A. formation	by breakdown of PhCr	by the two chemical reactions	external electrical energy
8	a 296 b 305 c 600	0.169 0.226 0.221	0.057	10.1	41.4	51.5	7.1
9	a 307 b 305 c 323	0.143 0.480 0.489	0.337	37.5	16.0	53.5	10.5
10	a 478 b 447	0.273 0.390	0.117	12.8	31.0	43.8	7.8
11	a 684 b 424 c 890	0.310 0.446 0.445	0.136	15.1	24.7	39.8	6.7
13	a 472 b 339	0.379 0.516	0.137	15.2	19.8	35.0	4.4
14	a 523 b 718	0.262 0.392	0.130	14.4	33.0	47.4	5.5
15	a 694 b 514	0.362 0.474	0.112	12.5	26.4	38.9	5.4
Average:			*0.147*	*16.8*	*27.5*	*44.3*	*6.8*

values since the differences between the two controls were without exception not significant.

The piece taken after stimulation was at first excised from the same region as that for the initial value. Since it appeared likely that the hole made by the first incision would change the current in the adjacent region, in later experiments two completely separated cuts were made. However, no difference became apparent between these two procedures. The time during which the discharges occurred was relatively short. In the first few experiments it was generally 3 to 5 min.; later it did not exceed 2 min. Since the

lactic acid formation is small and the rate of respiration low, the Q_{O_2} being about 0.3 to -0.4, it seems probable that during this period there is only a negligible resynthesis and that the amount of phosphocreatine actually split is not markedly different from that measured. This is supported by the result of Expt. no. 15 in which after 65 min. rest in water only two thirds of the phosphocreatine split during the stimulation period was resynthesized. The observations on the external electric energy released by 1600 discharges are summarized in Table 7 and compared with the energy released by the breakdown of phosphocreatine. The calculation of the energy of the phosphate bonds is based on the assumption that one mole of phosphocreatine yields 10,000 g.Cal.

Per gm. and impulse the average energy released by the breakdown of phosphocreatine is 32×10^{-6} g.Cal. The average external electric energy per gm. and impulse is approximately one fourth of this, being 8.2×10^{-6} g.Cal. It would be desirable to compare the energy released by the breakdown of phosphocreatine with the total electric energy produced by the organ instead of with the part only which was delivered to the external resistance. Any system which can be characterized by an electromotive force and an internal resistance will deliver maximum power to an external circuit having a resistance equal to the internal resistance. In this case the power spent in maintaining the current through the internal resistance is just equal to that delivered externally, so that the external power is half the total power. If the internal resistance is variable, as that of the electric organs probably is during the discharge, then it can not be matched at every instant by any constant external resistance. The maximum external energy will then be somewhat less than half the total energy. But measurements made on a number of oscillographic traces in the present experiments indicate that the ratio of the maximum external energy to the total will not be much different whether it is the internal resistance or the electromotive force or both which vary during the discharge. The reason for this is that the voltage rises rapidly to a maximum and then remains fairly uniform for most of the duration of the discharge, before falling rapidly at the end. Thus most of the electric energy is generated during a time when the electrical characteristics of the tissue are fairly constant, and consequently it makes little difference for the present purpose which characteristic is variable during the rest of the time. If therefore we could be sure that the external energy we measure is the only energy delivered outside the electric tissue, it would be possible to say that the total electric energy is about twice that measured externally and about half that released by the breakdown of phosphocreatine. But some energy must be spent in producing a current in the non-electric tissue, adjacent to the electric organs, and this escapes our present measurement. The conclusion from the observations made so far is that the maximum electric energy delivered to an external resistance is about one fourth, and the total electric energy at least half of that released by the phosphocreatine breakdown. Further experiments, however, are necessary to ascertain more definitely the ratio of

the total electric energy to that released by the breakdown of phosphocreatine.

D. *Lactic acid formation.* Heat production during nerve activity was demonstrated at a period—in 1926—when lactic acid formation was still considered to be an essential and primary process of muscle contraction. The first investigations on a possible formation of lactic acid connected with nerve activity were either negative or doubtful. Holmes, Gerard and Solomon (12) found a slight rise of lactic acid in frog's sciatic after prolonged stimulation (5 mg. per cent) but none in rabbit's nerve. Referred to the energy required the amount of 5 mg. per cent, if correct, is quite considerable. But with the chemical methods then used it is close to the limit of possible measurement. From experiments on nerves poisoned with iodoacetic acid, Feng concluded, in 1932, that formation of lactic acid, although not essential to nerve activity, is required if the nerve is to endure prolonged activity in oxygen (5). Addition of sodium lactate to poisoned nerve improved its capacity for prolonged activity in oxygen. The facts were confirmed by Chang and Gerard (3).

The formation of lactic acid as a result of the action potential was studied on the electric organ. In the experiments No. 8–15 described in the preceding paragraph the pieces of electric tissue while frozen were split and one part was used for the determination of phosphocreatine, the other for lactic acid. After 1600 discharges the amount of lactic acid formed per gm. of tissue was about 0.15 mg. on the average (see Table 7). The resting value varied between 0.15 and 0.35 mg. lactic acid per gr. If we assume that 1 mole of lactic acid formed releases 16,000 g.Cals. without neutralization heat, the energy supplied by the lactic acid formed is on the average 16×10^{-6} g.Cals. per gm. and impulse. In the 7 experiments summarized in Table 7 the external electrical energy released per gm. and impulse was on the average 6.8×10^{-6} g.Cals., the energy released by the breakdown of phosphocreatine 27.5×10^{-6} g.Cals. Thus the external electric energy amounts to only 15 per cent of that of the two chemical reactions. The actual ratio of external electrical to chemical energy is still smaller since at least the oxidation energy has to be added.

Since lactic acid formation is certainly a recovery process and could therefore occur at a delayed period, in a few experiments a third piece of tissue was cut 5–6 min. after the end of the stimulation period. The fish was continuously under control of the oscillograph and the piece was not taken if no more than a few discharges had occurred. Experiments No. 8, 9 and 11 show that no delayed lactic acid formation occurred during 5 to 6 min. after the end of stimulation. They also show that, at rest, no measureable lactic acid formation occurs during such a period.

Discussion

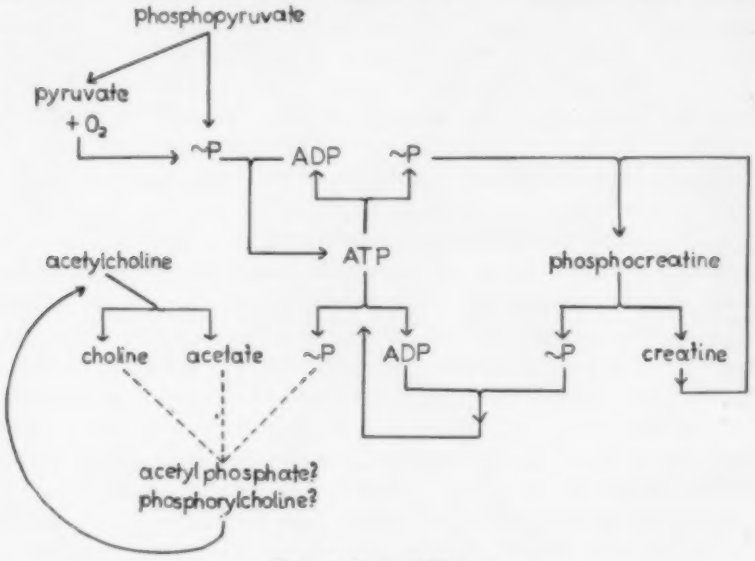
The experiments described above offer evidence that the breakdown of phosphocreatine is adequate to account for the electric energy released by

the action potential of the electric organ. The externally released electric energy per gm. and impulse is about 8 microcalories, the total electric energy at least twice as much, whereas the phosphocreatine split yields about 32 microcalories. The heat production observed in ordinary nerves is of the same order of magnitude as the energy changes observed in the electric organ. The total heat production in the crab nerve per gm. and impulse is about 35×10^{-6} g.Cals. In frog's nerve it is about $4\text{–}5 \times 10^{-6}$ g.Cals. at 0°C. and 10^{-6} g.Cals. at 20°C. Quantitative differences are always possible even if the process is fundamentally identical. The phosphocreatine content of nerve is markedly lower than that of the electric organ, although referred to the active surfaces the difference may be small. The heat production in electric tissue has not yet been measured. It will probably be somewhat higher than in crab's nerve since phosphocreatine breakdown and lactic acid formation already yield 44×10^{-6} g.Cals per gm. and impulse and the oxidation heat has still to be added. But the order of magnitude is still the same. Former observations suggest that the primary alterations during the action potential are connected with the release and breakdown of acetylcholine. The present observations are consistent with the conclusion that phosphate bonds may be used to resynthesize acetylcholine. It is not known how much acetylcholine is actually released during the discharge. But the amount which can be split during the duration of a discharge may give an indication of the amount possibly involved in the process, if we assume that acetylcholine is released during that period at a rate which is of similar order of magnitude to that at which it can be split. The duration of a single discharge is about 3 milliseconds. 1 gm. of electric tissue can split about 1 μg. of acetylcholine during that period, that is 5×10^{-6} millimoles. But it is not probable that this whole amount is actually released since it is reasonable to assume that the enzyme which has to remove the active compound is present in excess. The amount of phosphocreatine split per gm. and impulse is about 0.7 μg. or 3×10^{-6} millimoles. Thus the amounts of acetylcholine and phosphocreatine metabolized per gm. and impulse seem to be of the same order of magnitude even if we assume that choline esterase is present in a concentration of about 2–5 times in excess of that necessary to remove the acetylcholine released at a sufficiently high rate. This suggests that acetylation of choline occurs by means of an endothermic phosphorylation. Evidence showing that it is really so will be offered in the following paper.

The formation of lactic acid indicates that the phosphocreatine split may be partly resynthesized by the "Parnasreaction," i.e. by the transfer of P from phosphopyruvic acid, ATP acting again as intermediate. The lactic acid formation is however small and the greatest part of the energy required for the synthesis of phosphocreatine may be derived from the oxidation of pyruvic acid (or glucose).

Thus the following chain of reactions started by the breakdown of ACh, may be visualized (Fig. 1): ATP yields one phosphate for the resynthesis.

The adenosinediphosphate is immediately rebuilt to ATP by the breakdown of phosphocreatine. The creatine is rephosphorylated either by the phosphopyruvic acid, ATP acting again as intermediate ("Parnas reaction") or by the oxydative breakdown of pyruvic acid. Since the release of the ACh starts the whole chain it may be called the "acetylcholine cycle." It has still



Description of Figure

The "acetylcholine cycle"
ATP = adenosinetriphosphate
ADP = adenosinediphosphate
~P = energy rich phosphate bond

to be ascertained which may be the intermediate phosphorylated link between ATP and acetylcholine. One possibility is the formation of acetylphosphate, the compound described by Lipmann (15). Phosphorylcholine may also be the intermediate exchanging its phosphate with acetate.

Summary

Breakdown of phosphocreatine and formation of lactic acid as a result of the discharge were determined on the electric organ of *Electrophorus electricus*. The energy supplied by these two chemical processes was compared with the electrical energy released. The following results were obtained:

1. The external electrical energy per gm. of tissue and impulse is on the average 8.2×10^{-6} g. calories. The total electrical energy is at least twice as high. The amount of phosphocreatine split supplies per gm. and impulse on the average 32.8×10^{-6} g. calories, that is four times as much as the external electrical energy.

2. The amounts of acetylcholine and phosphocreatine metabolized as result of the discharge are of the same order of magnitude. This suggests that the energy of phosphate bonds is used for the resynthesis of acetylcholine.

3. The discharge leads also to lactic acid formation supplying an energy of 16.8×10^{-6} g. calories per gm. and impulse. The chain of reactions supplying the energy required to restore the resting condition of the electric organ thus appears to be fundamentally identical with those which are the source of energy in muscle contraction.

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THE FORMATION OF ACETYLCHOLINE. A NEW ENZYME: "CHOLINE ACETYLASE"

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INTRODUCTION

THE OBSERVATIONS described in the preceding paper (8) make it probable that the breakdown of phosphocreatine supplies the energy required for the restoration of the resting condition of the nerve membrane, adenosinetriphosphate being the intermediate link. Since the alterations at the neuronal surface during activity are almost certainly connected with release and inactivation of acetylcholine (ACh), and since the amounts of ACh and phosphocreatine which are metabolized as result of the action potential are of the same order of magnitude, it was suggested that energy rich phosphate bonds are used for the resynthesis of ACh. In this paper evidence is offered which shows that this assumption is correct.

METHODS

For the determination of ACh the method of Chang and Gaddum (3) was used in which the contraction of the frog's rectus abdominis in response to a known amount of ACh is compared with that produced by the solution with an unknown concentration of ACh. The muscle is suspended in a tube containing 8.0 cc. of frog's Ringer with $NaHCO_3$ in 0.0025 *M* concentration. Oxygen was continuously bubbling through. If the room temperature rose above 23°C., cooling was applied with water running through a glass cylinder and surrounding the tube which contains the muscle. The eserine concentration was 2.5×10^{-6} *M*. The rectus of the frogs used (*Rana pipiens*) is sensitive to 0.1–0.2 μg of Ach in 8.0 cc. However, 0.5 μg is near the optimal range, the curve drawn by the lever in two min. with this amount being about 4–5 cm. high. The determinations were therefore made mostly with the latter amount as standard. 0.4 and 0.6 μg of ACh gave distinctly different contractions, generally 2.5 mm. more or less respectively. Occasionally the muscle was less sensitive and higher amounts of ACh were used, up to 1 μg.

The solution in which the ACh had to be determined was prepared in the same way as by Quastel and his associates (7): 2.1 cc. were taken from the solution with the tissue suspension or from the extract in which the ACh formation was being studied. 0.5 cc. of 0.1 *M* phosphate buffer of pH 7.4 and 0.2 cc. of *N* HCl were added. The pH drops hereby to 2–3. After about 30 min. the solution was neutralized by addition of 0.2 cc. of *N* NaOH, centrifuged and the supernatant fluid was used for the test.

The solution to be tested was generally added in amounts of 0.1–0.2 cc. and sometimes for control in amounts of 0.3–0.4 cc. In such a small volume the interference of other substances, especially choline and potassium in higher concentration, was shown by repeated controls to be negligible. Only if inhibitory effects were expected higher amounts of the solution to be tested were used, 0.5 cc. or even more. A control was always run with the same amount of extract to which no adenosinetriphosphate (ATP) was added. ATP was prepared according to the method of Lohmann (6). Several modifications, however, were applied which facilitate the preparation and improve the yield.‡

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Results

According to Quastel and his associates acetylcholine is formed in brain slices under *aerobic* conditions only and if glucose or pyruvate is present (7). For the study of the mechanisms of enzyme reactions cell free extracts have to be used, as has been shown repeatedly. Experiments are presented in this paper in which ACh synthesis has been demonstrated in cell free extracts under *anaerobic* conditions. The mechanism involved as well as some of the properties of the newly isolated enzyme will be described in the following.

A. *Minced or homogenized tissue.* Formation of ACh in presence of adenosinetriphosphate (ATP) under anaerobic conditions was at first tested with

Table 1. Formation of acetylcholine (ACh) in finely minced or homogenized tissue, suspended in Ringer-bicarbonate solution in CO_2-N_2 atmosphere, in presence of adenosinetriphosphate. Choline chloride, sodium acetate and eserine sulfate were added

Exp. No.	Tissue	Kind of preparation	Addition	Duration min.	μg. ACh formed		
					per vessel	per gm.	per gm./hr.
1	frog's brain	finely minced	—	85	6.0	40.0	*28.0*
2	electric organ	finely minced	—	90	12.7	86.0	*57.5*
3	frog's brain	finely minced	—	100	2.0	14.6	*8.7*
4	frog's brain	homogenized	—	85	0	0	0
			fluoride 12×10^{-3} M	85	19.2	135.0	*95.0*
5	rat's brain	minced	fluoride 25×10^{-3} M	80	0	0	0
		homogenized		80	18.3	61.0	*46.0*

finely minced tissue. The tissue was suspended in 3.0 cc. frog's Ringer-bicarbonate solution in Warburg vessels. Two frog brains were used per vessel. The following additions were made: 1.2 mg. of choline chloride in 0.1 cc., 1.5 mg. of sodium acetate in 0.1 cc. and 0.6 mg. eserine sulfate in 0.1 cc. 0.2–0.3 cc. ATP, containing about 0.5 mg. of ATP-P_2O_5, were put into the side bulb. The vessel was then filled with gas mixture (5 per cent CO_2 and 95 per cent N_2). After thermoequilibrium was reached, ATP was added an the vessels were shaken for 80–100 min. A control was run without ATP.

The results were irregular. Some were negative, a few positive. Two of the experiments with finely minced frog's brain in which ACh formation was observed are recorded in Table 1. One experiment was made with finely minced electric organ. The rate of synthesis observed was higher than in frog's brain. The substance formed could have been phosphorylcholine. This

intermediate, however, does not affect the frog's rectus (2). Moreover, it withstands boiling with *N* NaOH at 100°C. for several hours (10). Addition of *N* NaOH completely destroys the substance formed in our experiments within 1 min., whereas boiling with weak acid, at pH 5.0, does not change the substance, as should be expected if the compound formed is ACh.

Since ATP does not penetrate the intact cell the possibility was explored whether ACh formation could be obtained in homogenized tissue in presence of ATP. In this case, however, ATP is rapidly split by adenosinetriphosphatase. Ochoa observed that fluoride inhibits adenosinetriphosphatase, but not the transfer of phosphate from ATP to a phosphate acceptor (9). If fluoride was added to the homogenized tissue a high rate of ACh formation occurred. Without fluoride no ACh was found (exp. No. 4). In presence of fluoride, synthesis was also observed in homogenized rat brain as shown in exp. No. 5, but not in minced tissue.

Since ACh formation occurred even after destruction of the cell, the experiments suggested that it might be possible to extract from the cell the enzyme performing the synthesis.

B. *Extraction of an acetylcholine forming enzyme from brain.* Extracts were prepared in the following way: 2–4 rat brains were cooled on ice, then ground with an homogenizer. During the grinding the tube was continuously kept in ice. 5–10 cc. of the solution were taken per brain containing KCl in 0.03 *M* concentration. Either sodium bicarbonate or phosphate were used as buffer in a concentration usually not higher than 0.01 *M*. Sodium acetate, choline chloride, eserine sulfate and ATP were added at the same concentration as described above. Sodium fluoride was always added, the final concentration being 25×10^{-3} *M*. 2.5–3.0 cc. of extract were used per vessel so that the final volume was between 3 and 4 cc. ACh formation was regularly obtained. The results are summarized in Table 2. The most active tissue examined was rat brain. 35–100 μg. of ACh were formed per gm. an hour. The lower value in exp. No. 1 may be due to the use of Ringer since Ca activates adenosinetriphosphatase. Cat and guinea-pig brain appeared to be less active. No activity was formed in liver and muscle extracts.

Presence of choline, eserine and fluoride appears necessary for an optimal rate of synthesis under the conditions used. If, on the other hand, no acetate was added, the rate was still the same or but slightly decreased. The following figures may be given as an example: In exp. No. 3 (Table 2) in which 62 μg. of ACh were formed per gm. an hour with all additions, no ACh was formed without eserine, only 9.6 μg. without fluoride, which is a decrease of 85 per cent. Without choline 20.0 μg. formed, a decrease of 68 per cent. Without acetate 66.6 μg. were found which is nearly identical to the value obtained with acetate.

The experiments show that brain and nerve tissue (electric organ) contain an enzyme which can be extracted and which in presence of the free energy of ATP synthesizes ACh. The enzyme will be called: "choline acetylase."

C. *Some properties of choline acetylase.—Stability.* The enzyme kept in the refrigerator loses in 24 hours about 50–80 per cent of its activity. Freezing did in some cases destroy the enzyme, in other cases the activity remained unchanged. The reason for this irregular behavior has not yet been found.

Influence of pH. Two observations were made so far, in one experiment with cat brain, the pH was changed by means of different phosphate buffers.

Table 2. Formation of ACh in brain extracts in presence of adenosinetriphosphate. Choline chloride, sodium acetate, sodium fluoride and eserine sulfate were added. Duration: 90 min.

Exp. No.	tissue	μg. ACh formed		
		per vessel	per gm.	per gm./hr.
1	rat brain	12.0	39.4	*26.3*
2	rat brain	52.0	150.0	*100.0*
3	rat brain	34.0	93.0	*62.0*
		34.0	93.0	*62.0*
4	rat brain	18.8	56.2	*37.5*
5	rat brain	40.0	105.0	*70.0*
6	rat brain	22.0	53.0	*35.5*
7	guinea-pig brain	11.2	27.7	*18.5*
8	pigeon brain	27.6	69.0	*46.0*
9	cat brain	11.1	17.1	*11.4*
10	cat brain	11.1	17.1	*11.4*
11	rat muscle	0	0	0
12	rat liver	0	0	0

In three different vessels the final pH was 6.6, 6.8 and 7.0 respectively, as determined by means of an electropotentiometer. 17 μg of ACh were formed per vessel, independent of the pH. In another experiment with rat brain the following formation of ACh was observed: At pH 7.5, 12.9 μg, at pH 7.1, 13.6 μg, at pH 6.5, 11.0 μg. These observations seem to indicate that the enzyme is not sensitive to variations close to pH 7.0 at which most experiments were carried out.

Effect of K^+, NH_4Cl and HCN. Quastel and his associates (17) found that ACh formation in brain slices in oxygen is increased by addition of K^+. When 0.027 *M* K^+ was added, the effect was optimal. The authors assumed that K^+ may act by increasing the cell permeability. No effect of K^+ was

Table 3. Effect of NH_4^+, Cu^{++}, iodine and iodoacetic acid (I.A.A.) on the activity of choline acetylase. Conditions as those in the experiments recorded in Table 2

Exp. No.	Compound added	Concentr. *M.*	time min.*	µg. ACh formed per vessel	Inhib. per cent
1	Control			40.0	
	Cu	16×10^{-5}		0	100
		3×10^{-5}		2.5	94
	NH_4Cl	2×10^{-3}		40.0	0
		4×10^{-3}		35.0	12
		8×10^{-3}		40.0	0
2	Control			13.5	
	I.A.A.	1×10^{-3}	20	0	100
	I.A.A. +pyruvate	1×10^{-3} 3×10^{-2}	20	0	100
	Iodine	1×10^{-4}	45	3.4	75
3	Control			22.0	
	Cu	1.5×10^{-5}		7.5	66
	I.A.A.	1×10^{-2}	60	0	100
4	Control			29.0	
	I.A.A.	2×10^{-4}	120	17.0	41
		1×10^{-3}	20	12.2	58
		1×10^{-3}	60	9.5	68
		1×10^{-3}	120	5.1	83
		2×10^{-3}	20	7.6	74

* Time of addition of compound before addition of ATP.

observed at a concentration varying from 0.02–0.06 *M*. This seems to indicate that the effect observed by Quastel is not directly connected with ACh formation but may be due—as assumed—to a permeability change.

Quastel *et al.* found under their experimental conditions a strong inhibitory effect of NH_4Cl on ACh formation at a concentration of 0.05 *M*. They concluded that NH_4^+, since it, like K^+ causes an increased permeability, has a direct inhibitory action on ACh synthesis. In extracts however, no inhibitory action was found as shown in exp. No. 1 of Table 3 when concentrations of NH_4Cl were used between 0.02 and 0.08 *M*. No effect was observed with cyanide in a concentration of 12×10^{-3} *M* and 4×10^{-3} *M*. This makes it improbable that choline acetylase contains an active metal group.

Iodoacetic acid. Barron (1), recently reviewing the enzymes containing sulfhydryl groups, stressed the significance of these groups since the protein

of a great variety of enzymes contains them. Iodoacetic acid (I.A.A.) acts specifically with −Sh groups as was shown by Rapkine (10, 11) and Dickens (4). ACh formation is strongly inhibited by sodium iodoacetate. One typical experiment is given in Table 3, No. 4. If the extract is shaken for 20 min. with a 1×10^{-3} *M* solution of I.A.A. the inhibition was already 58 per cent. With longer action of the compound on the enzyme a stronger inhibition was obtained. In another experiment the inhibition was 83 per cent with 5×10^{-4} *M* concentration of I.A.A. having reacted with the enzyme during 120 min., and 100 per cent with a 2×10^{-3} *M* concentration shaken for the same period.

It is possible that pyruvic acid is the precursor of acetic acid in the acetylation process. Since, therefore, inhibition of pyruvic acid formation by I.A.A. could be responsible for the inhibitory effect on ACh formation, pyruvate was added to the extract besides I.A.A. No effect of pyruvate was observed, the inhibition of ACh formation being as complete as without pyruvate (exp. No. 2). Sulfhydryl groups are highly sensitive to Cu. Choline acetylase is practically completely inhibited by a 3×10^{-5} *M* concentration of Cu and the inhibition was still 66 per cent at a 1.5×10^{-5} *M* concentration.

Iodine, which easily oxidizes −SH groups, had a marked inhibitory effect at 1×10^{-4} *M* concentration, if allowed to act on the enzyme for 40–60 min. An example is given in exp. No. 2. These observations make it possible to assume that choline acetylase may belong to the enzymes containing sulfhydryl groups. However, more evidence is desirable to support this assumption.

Discussion

Evidence is offered that an enzyme can be extracted from brain and nervous tissue (electric organ)—and apparently only from those—which synthesizes ACh in presence of ATP. It is generally accepted that ATP is the primary source of energy in muscle contraction. Engelhardt (5) recently made observations from which he concluded that this compound may be directly connected with the mechanical change occurring during muscular contraction. Direct evidence, however, is still lacking and the question whether contraction or relaxation of the muscle is linked to the effect of ATP is still open. The propagation of the nerve impulse is connected with electrical and not with mechanical changes. Strong evidence has accumulated that these electrical changes are connected with formation of ACh. The preceding paper indicates that energy rich phosphate bonds can supply the energy required by the action potential. The present paper shows that the free energy of ATP can in fact perform ACh synthesis as suggested in the "acetylcholine cycle."

The question still remains open what the phosphorylated intermediate compound is. Theoretically it could be acetylphosphate or phosphorylcholine exchanging the phosphate for choline or acetate resp. In this connection it may be of interest to know what the precursor of acetate is. There are several

possibilities which may be studied on dialyzed extracts. Many other problems remain open which require further investigations.

Summary

An enzyme has been extracted from brain and nervous tissue (electric organ) which forms acetylcholine. The formation occurs only in presence of adenosinetriphosphate (ATP). The enzyme is called choline acetylase.

The formation of ACh is greatly enhanced by fluoride which, according to Ochoa, inhibits adenosinetriphosphatase but not the transfer of phosphate to a phosphate acceptor.

K^+ at a concentration between 2 and 6×10^{-2} *M* and NH^+_4 at a concentration between 2 and 8×10^{-2} *M* do not affect the enzyme. Cu, iodoacetic acid and iodine have a strongly inhibitory effect. The implications of these observations for the mechanism of nerve activity are discussed.

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EFFECTS ON EEG OF CHRONIC LESIONS OF BASAL GANGLIA, THALAMUS AND HYPOTHALAMUS OF MONKEYS*

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IN A PREVIOUS COMMUNICATION (10) it was stated that injury to the cerebral cortical tissue of monkeys had no significant effect on the electroencephalogram (EEG), but that combined lesions of cortex and basal ganglia caused prompt and often permanent alterations in the pattern of cortical potentials. In the present paper it will be demonstrated that lesions restricted to thalamus and hypothalamus also have an effect on EEG in chronic conditions. In *acute* preparations like effects have been recorded by numerous investigators. Dusser de Barenne, Garol and McCulloch (4) produced changes in EEGs of monkeys by lesions in caudate nucleus and Dusser de Barenne and McCulloch (5) found changes in the postcentral cortical electrogram after lesions of the corresponding sensory nuclei of the thalamus and changes of EEG after strychninization of these postcentral areas. Dempsey and Morison (3) and Morison and Dempsey (11) similarly found focal changes in the parietal area after lesions of the thalamus of monkeys, and Obrador (12) abolished the EEG of cats by destruction of the hypothalamus. Acute decortication of monkeys under dial anesthesia, furthermore, changes the pattern of electrical potentials from basal ganglia and destruction within thalamus or hypothalamus slows the rate and lowers the amplitude of EEGs obtained from the surface of the cortex (7).

The present observations have been made on 52 chronic monkeys over a period of days, weeks or months before and after lesions of the subcortical nuclei. Such lesions have been made individually in the several nuclei or in combinations and with or without additional cortical ablations. Observations on the functional and anatomical changes of these animals will be published elsewhere (8).

METHOD

A Grass ink-writing 3-channel oscillograph was used. Records were taken by means of electrodes fastened to the skin of the scalp as described in a previous article (9). The same six combinations of leads from each side of the head in frontal, mid-parietal and occipital positions were used, but records from only three of these positions have been reproduced here as experience has shown that these three are the best for demonstrating both focal and general changes in the EEG following isolated ablations. The positions are shown in Fig. 1. The numerals for each position are then given in the succeeding figures which are reproductions of strips of EEG records. As will be seen, the leads 1–3 represent potentials obtained from the length of the left hemisphere, leads 4–6 are in similar position on the right, and leads 2–5 give intra-hemispheral potentials.

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The ablations were made aseptically, those from the cortex with a sharp-edged metal spatula and those of subcortical nuclei by gentle suction through a long-nosed glass sucker. The thalamus was reached after section of the corpus callosum; the hypothalamus from the base of the brain, approached rostrally above the optic chiasm. Various approaches to the

FIG. 1. Diagram of monkey's head showing positions in which EEG leads were applied.

basal ganglia were used as described in the protocols. The 52 monkeys used were either *Macaca mulatta* or mangabey (*Cerocebus torquatus atys*). Of these 14 were operated upon in infancy.

EXPERIMENTAL DATA

With one exception abnormal EEGs always appeared in animals with definite symptoms of central nervous system deficit, and the severity of the

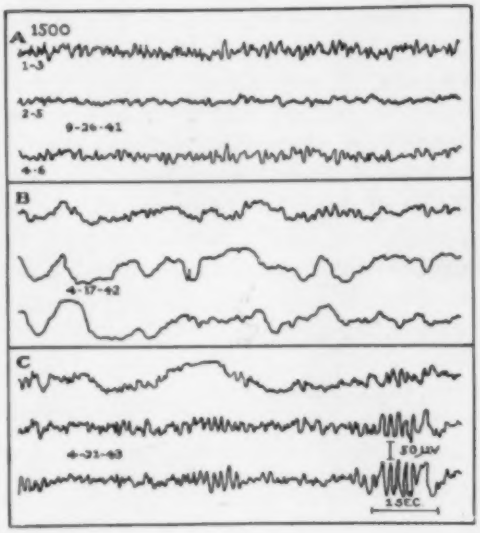

FIG. 2. Changes in EEG resulting from ablations of areas 6, 8 and caudate nucleus; A, normal record. B, after extirpations, left on Dec. 17, 1941, and right on Apr. 16, 1942; upper tracing (lt) shows a burst of high amplitude; lower tracings show irregular slow waves, as result of operation on preceding day. C, one year later, characteristic bursts of high amplitude present in all leads.

EEG disturbance was usually commensurate with the severity of the symptoms. Both were thus directly dependent on the size of the lesions within the basal ganglia since it has been shown (8) that there is little or no localization within the nuclei of the basal ganglia and that hence small lesions are im-

mediately compensated for by the remaining tissue both ipsilateral and contralateral to the injured area. Small lesions of caudate or putamen even if bilateral caused no alteration in EEG and this was true also in thalamus and hypothalamus. Although all subtotal lesions were followed by some recovery, large bilateral lesions resulted in permanent changes in EEG, or at least those which lasted more than a year after operation (Fig. 2).

The exception to the rule that size of lesion directly influenced degree and duration of both symptoms and changes in EEG was demonstrated in the 14 animals operated on in infancy. Such infants always showed minor symptoms as compared to older animals. But they had the most extreme and enduring changes in the EEG. Many of these, kept for two or more years after operation, grew to the size of others operated on later in life. They did not however develop severe symptoms of motor deficit although their EEGs remained very abnormal.

A. *Lesions of basal ganglia:* The observation made in a preliminary paper (10), that combined ablations from area 6 and caudate nucleus produced marked and permanent changes in EEG has been confirmed by the addition of more animals with similar ablations (11 in all). Figure 2 illustrates such changes. In A a normal preoperative record is given in which both medium 8 per second rhythm and fast waves of about 20 per sec. can be seen. On Dec. 17, 1941 the left areas 6, 8 and caudate head were removed. On April 16, 1942 the same areas were extirpated from the right side. Tracing B made the day after this second operation shows the characteristic changes which follow this procedure. On the upper tracing, from the left side, the rate of about 8 per sec. is normal and the shape of the waves nearly so although there is a suggestion of the periodic, high-amplitude bursts shown more definitely in C. The lower two lines of B, representing the right hemisphere and intrahemisphere potentials, show the marked slowing, unevenness and diminution in amplitude characteristic of a postoperative picture. In C, made a year later all three leads show abnormalities absolutely characteristic of injury to basal ganglia. The rate is again 8 per sec. and amplitude about that of the preoperative record. There are fewer of the fast waves, however, while the 8 per sec. medium waves are more regular and have periodic bursts of high-amplitude pointed waves like those of hypersynchrony in the human. In this animal, a mangabey of medium size, records were taken frequently for almost two years. During the postoperative period the only change was in the rate, which was slowed by the operation and then returned to preoperative levels. This animal has been rigid and has marked tremor which is unabated now more than a year after its last operation.

Figure 3 shows similar changes from the records of a mangabey which had had caudate and putamen removed on June 23, 1941, the left area 8, on September 15, 1941 the same areas from the right; and on April 13, 1942, section of the right ansa lenticularis. In A, the unilateral removal of cortex, caudate and putamen has done little to the EEG unless a certain amount of irregularity is present. In B, within the week after the second operation.

there is much more irregularity and some slowing of rate. Six months later, in C, the record shows extreme "fencing" or hypersynchrony, as it had throughout the intervening time. Ten days before the record in D, the right ansa lenticularis was sectioned producing much more marked irregularity although the "fence" arrangement and the absence of fast waves persists. This last finding occurred in all five animals in which the ansa lenticularis was sectioned either unilaterally or bilaterally.

Lesions anywhere in the basal ganglia if they were large enough produced the same effect as those shown in Fig. 2 and 3. The irregular bursts of high amplitude, regular potentials are characteristic of such disturbances. There

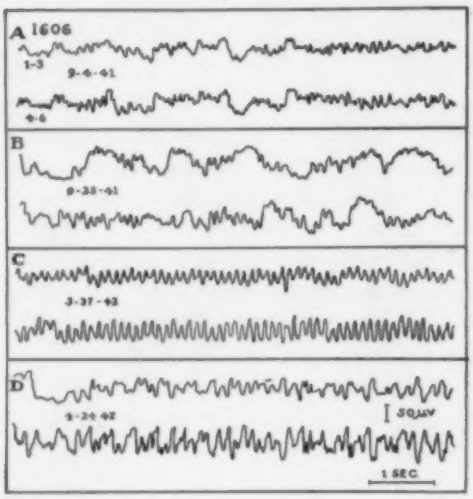

FIG. 3. Effect of ablation of area 8 and caudate (B and C), and of section of ansa lenticularis (D). Note regular high-amplitude rhythm in C and irregularities in D (see text).

was a suggestion that hypersynchrony was more intense following lesions in which caudate was injured and that irregularity of pattern and large, high amplitude waves were found more often from injury to ansa or globus pallidus. Often, however, it would be impossible to distinguish between the records of these three lesions.

Figure 4 has excerpts from the records of the left and right hemispheres of a monkey from which portions of the putamen and globus pallidus were removed from an approach via the base of the brain, the left on Feb. 22, 1943 and the right on March 22, 1943. The normal record is given in A. In B, three days after the first operation, the left, operated side has waves of low amplitude and slow, those of the right are higher and somewhat irregular. Six days later in C when the effect of operation has worn off, both sides show slowing and definite irregularity but the rate on the right is still faster than that on the left. In D, 12 days after the second operation, there is a characteristic picture; many slow high-amplitude waves appear in both sides. The lesion at autopsy is shown in Fig. 6.

The tendency to irregularity and variability of pattern which was present in these animals with lesions of the basal ganglia was demonstrated in another way, namely by the occurrence of epilepsy after operation in a great many cases. It happens occasionally that, following operation upon the motor cortex, epilepsy appears during the first postoperative week but this is extremely rare. It was a common symptom when the basal ganglia together with cortex were injured. It appeared less often when basal ganglia alone were damaged. Since it was periodic and transient there are no valid statistics

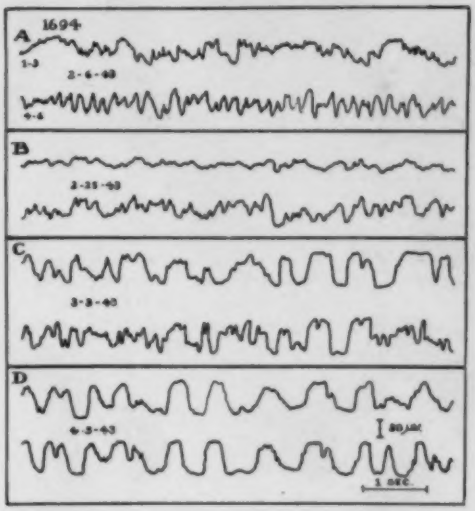

FIG. 4. Changes in EEG following ablation from left (B) and right (C) putamen and globus pallidus (see text).

as to its incidence, for clinical epilepsy was seen in a number of animals at times when no record was taken, and may have been missed in as many more. On the other hand, sub-clinical seizures were not infrequently recorded in animals which had never been seen to have grand mal attacks and appeared on the records of some animals which had been known to have attacks but which at the time of the EEG seizure, appeared normal clinically. All this epilepsy occurred within a few weeks after operation and disappeared then, never to reappear. It is probable therefore that it had to do with tension and edema of the tissues which were disturbed at the time of operation. The reason for its high incidence in combined cortical-subcortical lesions and not in those limited to cortex may be due merely to this edema, for there was, of necessity, more distortion of motor pathways in the combined cortical and subcortical lesions.

B. *Lesions of thalamus:* Lesions of the thalamus were made in five monkeys. In each, the approach was via a sectioned corpus callosum. In each large lesions were made involving either the entire massa intermedia (1 case) or most of one side of the thalamus (3 cases). One animal had bilateral ablations from this region. No attempt was made to produce small lesions restricted to one or more nuclei in this series because it had been found previ-

ously that such small lesions of thalamus as of basal ganglia caused no discernible effect on the EEG obtained from the surface of the scalp.

Figure 5, A, B, and C show the changes which occur following such thalamic lesions. On Oct. 26, 1942 a large excavation was made from the thalamus after section of the overlying corpus callosum. On January 22, 1943 the hypothalamus was nearly completely destroyed. The normal clearly defined 10 per sec. waves seen in A have changed to slow wavering irregular rhythm in B ten days after the lesion was made in the thalamus. Three months later, the rate has increased to about 8 per sec., which is not quite that of the preoperative record, but there was still marked irregularity and a wavering baseline. This irregularity and tendency to change was probably the most noticeable characteristic of the monkeys with thalamic injury. There was always, in addition, a slowing of rate, and frequent appearance of large slow hill-like waves such as are shown in both B and C.

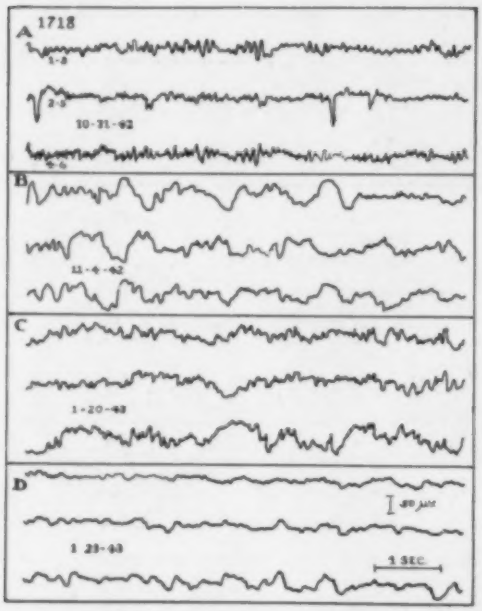

FIG. 5. Effect of lesions of thalamus (B and C) and hypothalamus (D) (see text).

C. *Lesions of hypothalamus:* Records from animals with lesions of the hypothalamus have been made in five cases, and in each, the changes were similar, although the clinical symptoms varied. Two of these, one of which was the last described animal above, which had already had a lesion of the thalamus, developed, after the hypothalamic ablation, a profound "pathological" sleep such as has been described by Ranson (14) in monkeys. These two (Fig. 5D and 7) had the most pronounced changes in EEG, the other three had changes of the same nature but of less severity. In all the amplitude of EEG diminished almost to zero, the rate slowed and almost nothing could be found which resembled the normal EEG pattern. In contrast sleep in these monkeys, either normal or when induced by luminal causes the same changes as in man, and which are the opposite of those which followed hypothalamic injury. In sleep the amplitude becomes greater, the rate more variable, but often very fast, and the entire picture is one of accentuation of the normal alpha rhythm. In Fig. 7 is shown the record of one of these somnolent monkeys five days after the lesion was made which is shown in Fig. 8, and three days before its death from hypostatic pneumonia, the result probably of its extreme inertia.

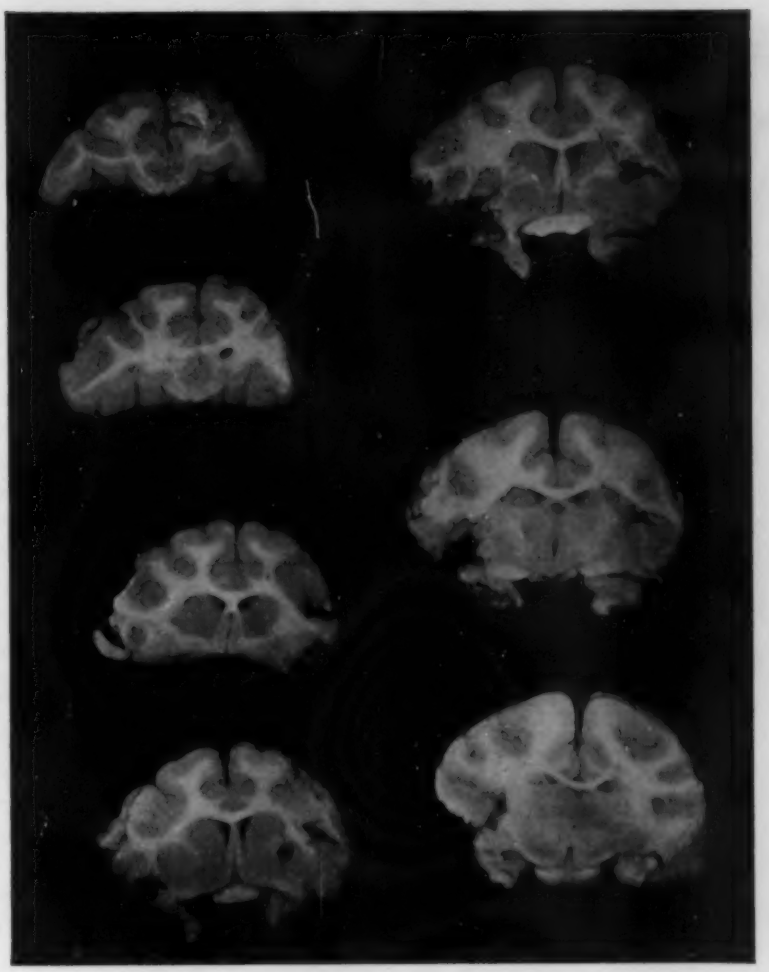

FIG. 6. Bilateral lesions of putamen and globus pallidus of monkey, whose record is shown in Fig. 4 (No. 1694).

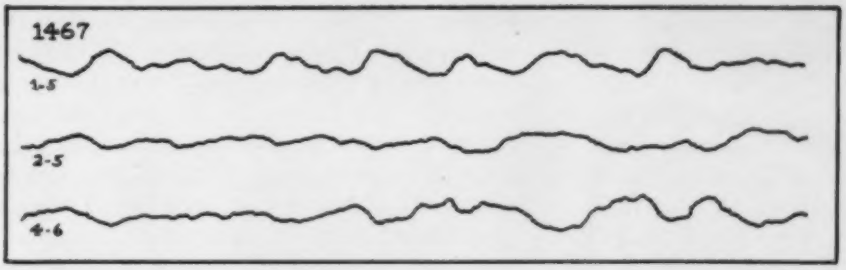

FIG. 7. EEG of somnolent monkey with lesion of hypothalamus shown in Fig. 8.

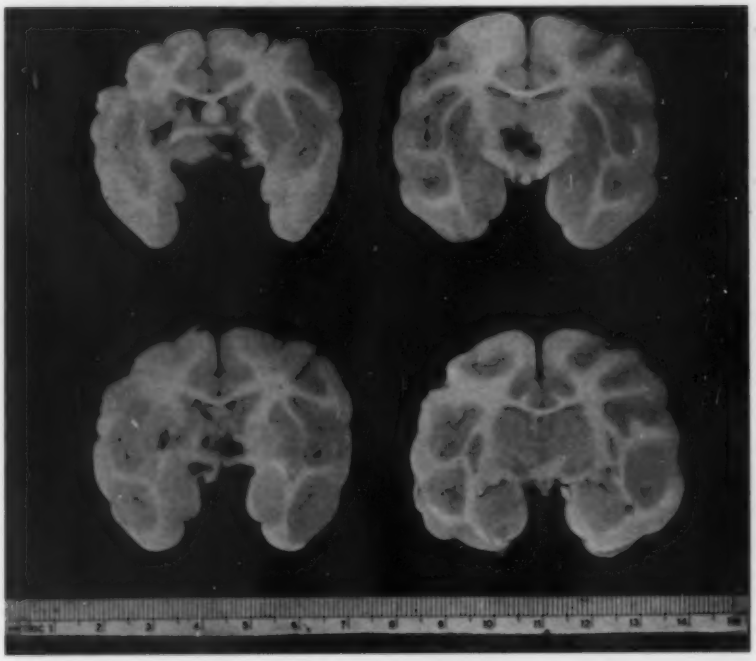

FIG. 8. Lesion of hypothalamus producing EEG record shown in Fig. 7.

DISCUSSION

The absence of abnormal EEGs in monkeys after lesions restricted to the cerebral cortex, although they appear when the sub-cortical nuclei have been injured is not consistent with the usual present-day concept of the EEG which has been shown both clinically and experimentally to be directly concerned with cortical excitability. Nevertheless there is abundant recent evidence which relates the subcortical areas as well to the EEG.

The absence of changes from cortical injury in monkeys can be explained by several factors. First, the size of the monkey's head is so small, that when obtaining records from the scalp and not directly from the surface of the brain no localization, or at best only lateralization appears. Since in man, nearly all clinical localization today is made by comparing the EEG from one area with that of another (2, 6) and this factor is not useable in the monkey small changes could not be detected in the latter. To this must be added the fact that the "normal" EEG even in man is only the usual for that patient as compared to what have already been established as standards and that the material for standards in the monkey at present is much less. That acute changes of record can actually be obtained in monkeys by cortical excitation is well known, and Pacella, Barrera and Kopeloff (13) have shown that chronic irritation of the cortex can produce recordable episodic seizures. This

was done by placing irritating substances on the surface of the cortex. Apparently the reasonably cleancut extirpation has no such irritative effect.

There is recent clinical evidence that primary subcortical lesions may be the cause of epilepsy and hence of abnormal EEGs in man. Foerster and Penfield (6) have discussed what has since become a well-recognized clinical fact, that trauma to the brain, when deep, as in penetrating wounds, frequently produces epilepsy as a late manifestation and that this is due to traction on the cortex by the formation of scar tissue. Since there can be no doubt that epilepsy is a manifestation of cerebral cortical irritation, and since it was noticeably frequent in these animals with lesions of the basal ganglia, it is probable that disturbance of the cortico subcortical pathways from the motor area with consequent pressure or traction on the excitable motor cells must have been at the basis of the epileptic attacks and of at least some of the abnormal records which were not accompanied by seizure.

It is noteworthy, however, that lesions of neither thalamus nor hypothalamus caused epilepsy but several additional facts must be considered in this respect. First, it happened that the ablations of these latter two groups did not have concomitant lesions of cortex as did many of those of the basal ganglia which produced epilepsy; second, neither thalamus nor hypothalamus are so near the internal capsule and corticospinal tracts as are the basal ganglia; third, since the basal ganglia are functionally more intimately related to motor performance this may be the reason that their disturbance produces epilepsy; fourth, the caudate nucleus, at least, is known to be directly related to the suppressor function of area 4s of the cortex (4) and if removal of suppression is one cause of epilepsy this may conceivably play a part in the type of abnormal EEG which was found. Distortion of the subcortical tracts would be as likely to appear after thalamic and hypothalamic lesions as after those of basal ganglia for, in our experience in the animals that came to autopsy some days after operation, there was as apt to be edema of the tissues in one lot as in the other.

The presence of abnormal EEGs in patients with chorea has been now established. In two recent articles (1, 15) the changes described in the EEGs of a series of children with Sydenham's chorea, which is known to be a disease affecting basal ganglia primarily, are strikingly like those found in the present series.

Both groups of authors stress the constancy of abnormal EEGs in this condition and furthermore the relation, both clinically and from electroencephalographic evidence, between chorea and epilepsy. The children of the series of Usher and Jasper all had generalized abnormalities of EEG, consisting of a decrease or absence of the alpha rhythm and presence of continuous slow delta wave activity of high amplitude. Slowing of rate and increased amplitude were the most conspicuous changes in the monkeys. In addition, the more severe the chorea the more gross the changes in EEG in the children, and improvement in EEG was always correlated with clinical recovery. The authors state that the changes in EEG of chorea are not specific for this

condition and that they resemble certain dysrhythmias found in epileptics between attacks and also those found following acute head injury.

Buchanan, Walker and Case (1), confirm these observations and stress the similarity between the changes in epilepsy and in chorea. They consider that the families of children with chorea, like those with epilepsy, show changes which are of the same nature although less pronounced than those of the patients. It is possible that the changes found in both man and monkey following acute head injury may be dependent on injury or distortion of the basal ganglia also. Evidence at present is strongly in this direction. For, neither in man nor monkey can the changes which occur acutely in the two conditions be told apart. It is well known also that the basal ganglia are particularly susceptible to minute petechial hemorrhage following trauma which might well be the underlying cause of the disturbance.

The relation of the hypothalamus to sleep has long been known but its exact influence on and interconnection with the cortex are still not clear. Both the work of Obrador (12) and ours on acute preparations (7) is borne out by the present findings that destruction of the hypothalamus diminishes or abolishes the pattern of cortical potentials. How this relates to the apparent "release of function" exerted by the normal hypothalamus during sleep is difficult to imagine.

Summary

1. In monkeys chronic lesions of the subcortical nuclei have been found to produce changes in the EEG although lesions restricted to cortical tissue cause no such change.

2. Lesions of the basal ganglia if large enough, or of basal ganglia and cerebral cortex cause permanent alteration in the EEG.

3. Epilepsy, either clinical or subclinical and detectable by EEG, was a frequent finding following lesions to basal ganglia.

4. The changes of EEG following lesions of basal ganglia can be directly correlated with the functional changes in the monkey and are similar to those seen in human children with chorea.

5. Lesions of the thalamus caused marked slowing of rate, irregularity of pattern and the appearance of high, slow rounded waves at frequent intervals.

6. Lesions of the hypothalamus caused great slowing of the rate and diminution of amplitude. With large destruction practically no pattern of potentials remained.

7. This is in direct contrast to the effects of sleep which are to increase amplitude and intensify the normal pattern.

8. It is suggested that the post-traumatic changes which appear in both man and monkey may be directly related to changes within the basal ganglia.

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HAND AND FOOT PATTERNS OF LOW ELECTRICAL SKIN RESISTANCE: THEIR ANATOMICAL AND NEUROLOGICAL SIGNIFICANCE*

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IN A PREVIOUS PAPER it was shown that sympathectomized areas on any part of the body can be sharply defined by means of the electrical skin resistance method (1). These areas, which do not sweat, have a higher resistance than the surrounding normal areas. In sympathectomized patients these patterns of high skin resistance, which agree closely with the distribution of the sensory dermatomes, come out most clearly after the entire body has been heated in a hot air cabinet—the temperature used being that which in the normal person causes a general decrease in skin resistance to a low level with the disappearance of all patterns. At ordinary room temperature, most normal persons manifest well defined areas of low resistance on the face, hands, feet, axillae, and antecubital fossae (2). When an individual is immersed in a hot air bath, the facial area enlarges until it includes first the entire face, then the head and neck, and finally the entire body. Both cooling and sleep cause a marked constriction to a very small area which finally includes only the lips. The area of low resistance most commonly present in normal individuals at room temperature coincides closely with the hairless area of the skin on the face of gorillas and chimpanzees and other primates. The patterns of the areas of low skin resistance coincide closely with the patterns of areas of remaining sensitivity found on the faces of patients with syringobulbia and, like them, vary concentrically around the mouth.

In the following experiments detailed examinations were made to determine the shape and distribution of the areas of low skin resistance on the hands and feet under varying conditions. Are these areas fixed or do they also vary with external temperature and sleep? Do the patterns correspond to the distribution or activity of the sweat glands or peripheral blood vessels? Do the patterns show any relation to the peripheral nerves, sensory dermatomes, or central or autonomic nervous system anatomy?

METHODS

Previous papers contain a full description of the technique (1, 2). By means of a small ear clip, one electrode, a ½ inch zinc disc, is clasped to the ear lobe—the skin of which has been pricked with a hypodermic needle to eliminate the resistance of the skin under this electrode. A paste made of kaolin and saturated zinc sulphate solution establishes contact between this electrode and the skin. The movable electrode, which consists of a small ¾ inch zinc disc fastened at right angles to the end of an insulated zinc rod, is held by the operator and can thus be touched against the skin on any part of the body. No paste is used with this electrode. The dermometer used for these experiments consists of a 4.5 V. battery, a

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microammeter, and a variable resistor. The instrument is used only to register differences in skin resistance, not to obtain actual measurements.

The following procedure is used in mapping areas of high and low skin resistance. After the fixed electrode has been attached to the ear, the instrument is set by means of the resistor so that a moderate difference in potential exists between the electrodes. As the movable electrode is pressed lightly against the skin on different parts of the body, the ammeter is watched closely for sharp changes in the amount of current flowing through the circuit. This amount depends largely on the resistance of the skin under the movable electrode. When the resistance is high, little or no current flows and the indicator on the dial does not move; when it is low, the current that flows is strong and the indicator moves quickly across the face of the dial. The surface of the skin is explored with the movable electrode until a point is found at which the resistance shows either a sharp rise or fall. This point is then marked with a black skin pencil. Adjacent areas are explored until another point of marked change in skin resistance is located. Successive points thus located are joined together with a pencil line and the process is repeated until finally areas of high and low skin resistance are completely defined; that is, until the two ends of the line joining the points come together. Seventy normal adults and ten new born infants were used in these experiments.

Results

A. *Hands.* Figure 1 shows the two most common patterns of the areas of low electrical skin resistance found on the palmar and dorsal surfaces of the hands of normal individuals. The pattern shown in Fig. 1A (stippled area) includes all of the palmar surface as well as the dorsal surface of the two distal phalanges of the fingers and thumb. The patterns shown in Fig. 1B include most all of the palmar surface but none of the dorsal surface.

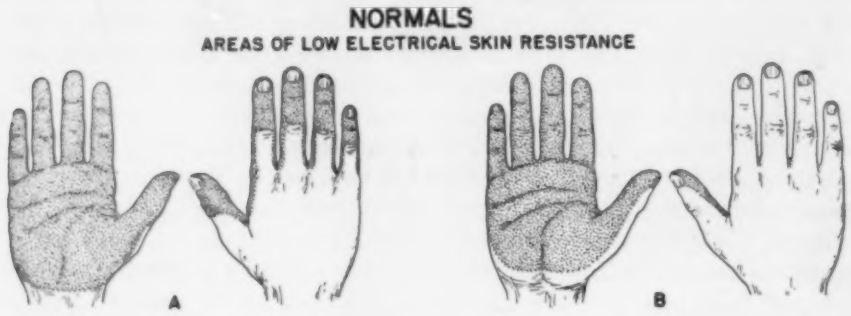

Fig. 1

The resistance on the outside of the closed area was usually about 4 times higher than on the inside, while the line of separation between the areas of high and low resistance was scarcely more than $\frac{1}{8}$ of an inch in width. This line of demarcation was quite as sharp as between areas of sympathectomized and normal skin described in a previous paper.

Effect on hand patterns of cooling and heating the body. Figure 2 gives a typical record of the effects produced on the hand pattern of low electrical skin resistance by cooling and heating the body. In these experiments the subjects were immersed to the shoulders in a continuous tub bath; the hands and arms rested on the sides of the tub and remained dry and accessible for the skin mapping. Under normal conditions, that is, at room temperature,

the pattern of low resistance of this subject included all of the palmar surface and none of the dorsal surface. After 2 minutes in a bath at 75°F. the area of low resistance on the palmar surface became constricted and divided

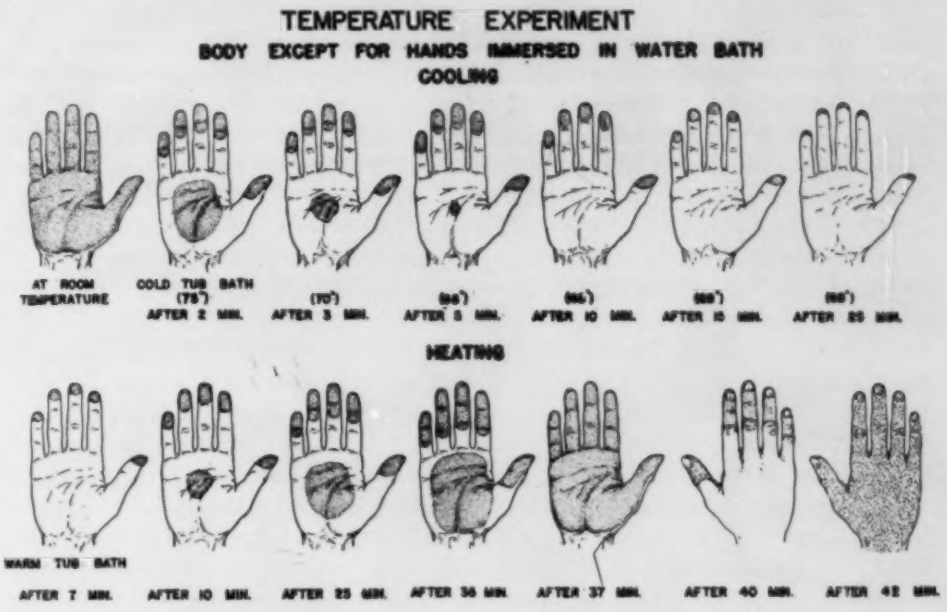

FIG. 2

into small areas, one on the tip of each finger and thumb, and another, larger one, in the center of the palm. Progressively, with time and with the lowering of the temperature of the bath, these areas grew smaller until finally after 25 minutes' immersion when the bath had cooled to 68°F., they included only the tips of the fingers and thumbs. At this time hot water was run into the tub gradually.

After 7 minutes the areas on the tips of the fingers had enlarged slightly. After 10 minutes the palm of the hand again showed a small area of low resistance and the areas on the tips of the fingers had become larger. During the next 20–25 minutes the finger, thumb, and palmar areas gradually increased in size until they became consolidated again into a large area which cov-

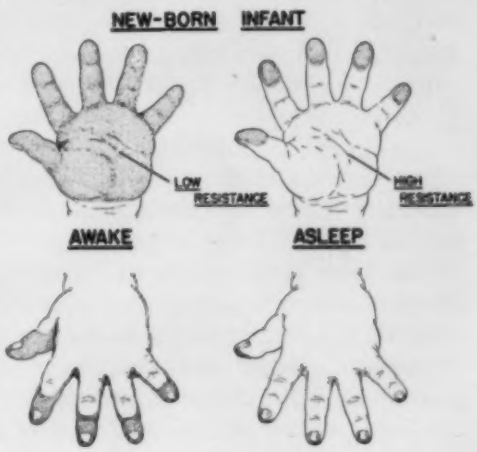

FIG. 3

ered the entire palmar surface. With longer heating the area of low resistance spread further, including the dorsal surface of the fingers and thumb up to the second joint, the entire hand, then glove shaped patterns, and finally the entire arm. Ten other subjects gave essentially similar records.

Changes in hand patterns during sleep. In a previous paper it was reported that during sleep the facial area of low skin resistance contracts markedly. In the present experiment we found that the hand patterns also become very much constricted. These observations were made on newborn infants. Figure 3 gives a typical record. During the waking state this baby's area of low resistance included the entire palmar surface and all of the dorsal surface

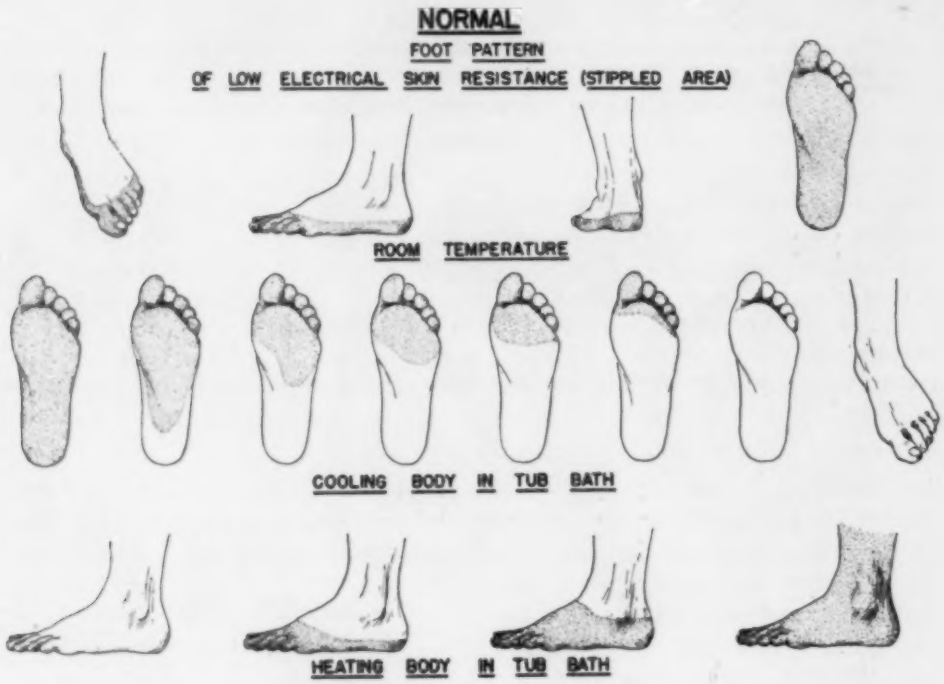

FIG. 4

of the finger and thumb to the first joint. During sleep one half hour later this area had become constricted until it included only the tips of the fingers and thumb. Ten babies gave similar records.

B. *Feet.* The top row in Fig. 4 shows the foot pattern of low electrical skin resistance found most frequently at room temperature in normals. It includes the entire plantar surface and a narrow band, about 1 inch in width, which runs around the foot laterally and up over the dorsal surface to include part or all of the dorsal surface of the toes. Less frequently the area includes only the plantar surface and none of the lateral or dorsal areas.

Effect of temperature changes on foot patterns. In most individuals cooling

of the body in a tub bath of 68°F. caused the pattern of low electrical skin resistance to shrink until it included only the spaces between the toes, while heating the body caused the pattern to enlarge first to include only the plantar surface, and a narrow band around the foot, then successively slipper and sock shaped areas, and finally the entire leg. The second and third row of feet in Fig. 4 show fairly typical changes.

Relation of patterns of low skin resistance to sweat glands. These observations showed that under normal conditions the areas of low electrical skin resistance on the hands of normal individuals include all of the palmar surface. It will readily be seen that this area agrees closely with the areas of skin having a rich supply of sweat glands, the so-called porous areas which have a different anatomical appearance from the shape of the back of the hand, not only in structure but in color. Since these areas may often show active sweating while the skin on the backs of the hands is dry, differences other than the number of sweat glands probably distinguish the palmar from the dorsal surfaces of the hands. In moving the electrode from the palmar to the dorsal surface the sharp line of change in resistance usually occurs near the mid-line on each finger, where the porous area of the skin ends, though the low resistance area often includes the dorsal surface of the last two phalanges of the fingers and thumb, as was shown in Fig. 1A. This pattern is often obtained in normal individuals under ordinary conditions, and is always found during any kind of emotional excitement or tension. It is not known whether the skin on the dorsal surface of the last two phalanges has a richer supply of sweat glands than the skin on the remainder of the dorsum of the hand. There is on the feet also a close correspondence between the most common pattern of low skin resistance and the porous areas of skin. Here also even under ordinary circumstances the pattern may extend up over the two distal phalanges of the toes, or even up over the entire toes, and up over the edge of the foot.

Relation of patterns of low electrical resistance to distribution of blood vessels. Anatomically there appears to be very little relation between the skin resistance patterns and the distribution of the blood vessels beyond the fact that the palmar surfaces have a richer supply of blood vessels. In 12 individuals we recorded the patterns before and after complete occlusion of both venous and arterial supply to the hands for as long as 15 minutes and found no consistent changes. In some subjects the patterns contracted until the low resistance area included only the tips of the fingers, just as during sleep or in cold temperatures, while in others, the patterns became enlarged to include not only the hands but also part of the arms. The pain from the hands and arms may have contributed to the enlargement of the patterns.

Relation between patterns of low electrical resistance and hair distribution. On both the hands and feet, the patterns of low electrical skin resistance under standard conditions were similar to the patterns of hair distribution, having the same shape, but being smaller. The low skin resistance area over the dorsal surface of the last two phalanges of the fingers and thumb, which

in most individuals, primates as well as human beings, is hairless, is particularly suggestive of a relationship between these two phenomena.

Relation of patterns of low electrical skin resistance to sympathetic nervous system. In observations made on over 50 patients with upper thoracic or lumbar sympathectomies we did not find any pattern of low resistance on hands or feet, even after the patient had been well heated in the hot air cabinet. This would indicate then that the areas of low resistance depend at least in large part on sympathetic innervation of the extremities. In agreement with this conclusion is the observation that the hand and foot patterns become enlarged under conditions in which the sympathetic nervous system becomes activated, such for instance as strong muscular effort or emotional excitement, and become constricted under conditions in which the sympathetic nervous system becomes inhibited, such for instance as sleep and inactivity.

Relationship of low skin resistance to distribution of nerves. Before discussing the possible relationship between the skin resistance and nerve distribution patterns we must recall some of the most important features of the skin resistance patterns. In the first place, they seem to correspond roughly with sections made perpendicularly to the axis of the arms and legs. When the areas become enlarged they proceed up the extremities in glove and sock patterns; when they become smaller they gradually recede to the most distal ends of the axes, the tips of the fingers and toes. Clearly at no stage do the skin resistance patterns coincide with the distribution of any of the peripheral nerves, such for instance as the ulnar or radial nerves to the hands, or the tibial or peroneal nerves to the feet. Likewise the skin resistance patterns do not coincide with the distribution of any of the segmental nerves. They may agree more closely with the distribution of *sympathetic* nerve patterns from the cerebral cortex since the work of Gutterman and List and others has shown that sympathetic functions like the motor or sensory functions of the legs, arms, and head may be represented in the cerebral cortex (3). The skin resistance patterns seem to include the entire hand and foot, not segmental or peripheral divisions.

Discussion

The results of this study demonstrate that in normal individuals the skin on the hands and feet, just as on the face, shows hitherto entirely unsuspected patterns of low electrical resistance. Sharp lines, less than $\frac{1}{8}$ inch in width, usually separate the areas of low from the areas of high resistance. In most instances the resistance in the low areas is about one-fourth as high as in the surrounding areas. It is especially noteworthy that these great differences in resistance exist without any visible differences in the skin. In the previously reported experiments it was found that the facial patterns of low electrical skin resistance coincided with cross sections of the face made perpendicular to the somatic axis of the body and head; that is, regarding the mouth and lips to be the most forward end of the axis in man as well as

animals. It appeared that the patterns correspond closely with the axial segments.

The patterns on the hands and arms and on the feet and legs seem to represent analagous cross sections along the axis of the arm and leg respectively. As the patterns become constricted, they move toward the ends of the axes; that is, to the tips of the fingers and to the ends of the toes, just as the patterns on the face moved toward the mouth. As the patterns enlarge, they move up the axes of the arms and legs in more or less circular patterns. These results suggest as we pointed out above, that these patterns must depend on some fundamental neurological mechanisms such as are found only in the higher centers of the brain stem or cortex. The facial areas would thus be more likely to be associated with such cortical areas than, as was suggested in the previous paper, with the trigeminal nucleus.

It is noteworthy at this point to call attention to the similarity in shape between the sock and glove patterns of low electrical resistance and the patterns of anaesthesia or hyperalgesia often found in hysterical patients. The finding of the skin resistance patterns indicates that there may be an anatomical basis for the sock and glove patterns of the hysterics. The presence of sock and glove patterns of anaesthesia in peripheral neuritis, has already established the existence of such patterns. We have not determined whether these patterns found in the vitamin B_1 deficient patients correspond with the skin resistance patterns.

The mapping of the areas of low skin resistance may help to throw some light on the mechanisms involved in the production of various pathological conditions of the extremities, such as are seen in Raynaud's disease, in scleroderma, diabetes, etc. It may also be of help in the study of the effects of low and high temperatures on the body. In preliminary studies on the effects of heat made during prolonged summer heat spells, we found that in many instances the hand and foot patterns became reversed; the areas of low resistance became in a relative sense the areas of high resistance, that is, the skin on the rest of the body had a lower resistance than that on the palmar and plantar surfaces. The mapping of the hand, foot and facial patterns may also be useful in the study of acute and chronic emotional disturbances seen in psychiatric patients.

Summary

1. Under normal conditions, that is, at ordinary room temperature, etc., the hands and feet, like the face, show sharply defined areas of low electrical skin resistance. On the hands these patterns usually include the entire palmar surface up to the line which divides the dorsal and ventral parts of the hand. The skin of this area shows a resistance about one-fourth that of the skin of surrounding areas. On the feet the areas of low electrical skin resistance usually include the entire plantar surface and a small band along the side of the foot and over the toes.

2. These areas become constricted in cold temperatures and during

sleep, and become enlarged in warm temperatures and with exercise or excitement. When the patterns contract, the tips of the fingers and the toes are the last to show a low resistance. When they expand, the patterns first envelop all of the dorsal surface of the hands and feet and then move up the arms and legs, showing regular sock and glove patterns.

3. The possible relationship of these areas to the distribution of sweat glands, blood vessels and hair was considered.

4. It was shown that the patterns do not conform to the distribution of any of the peripheral nerves or the sensory dermatomes. It was suggested that they might represent cortical or sub-cortical patterns of the distribution of sympathetic nerves to the extremities.

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NATURE OF PARESIS FOLLOWING LATERAL CORTICO-SPINAL SECTION IN MONKEYS*

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A TRANSIENT contralateral spasticity, consisting of hypertonus, principally extensor in the lower extremity and flexor in the upper, with accompanying hyperactive deep reflexes and clonus, was observed by Hines (2) following ablation of Area 4s (the "strip area") in the rhesus monkey. As yet lesions placed in other areas of the precentral cortex or those placed in the pyramids (4) have failed to give this syndrome. Subsequent to sectioning of the pyramids, Tower (4) observed a paresis characterized by hypotonus, hypoactive deep reflexes, and no evidence of clonus. In the present investigation a similar paresis has been found to exist after the interruption of the lateral cortico-spinal tract at cervical or thoracic levels of the spinal cord.

METHODS

In a series of 9 *Macaca mulatta* monkeys, dorso-lateral chordotomies were performed, in five instances at cervical (C-4) and in four at thoracic (T-9) levels. Exposure was obtained by a laminectomy and the cord incision was made from the postero-lateral sulcus downward and lateralward to the origin of the dentate ligament.

The results of neurological examinations previous to and at routine intervals after the operation were recorded.‡ The pre- and post-operative behavior of the monkeys in their cages was also noted. With the exception of one case, still under observation, the animals were sacrificed within four to nine weeks. Sections through the lesion were prepared with the Weil stain and those above and below the lesion, by the Marchi technique. Projection drawings of the lesions are shown in Fig. 1. It should be noted that the lesions involved other tracts of the dorso-lateral column in addition to the lateral cortico-spinal tract.

RESULTS

In examining the extremity or extremities of the operated side below the level of the lesion, results were recorded in degree of response compared with the response obtained from the corresponding contralateral extremity subjected to the same test. As the extremities of the opposite side presented no apparent changes after operation, this served as a control in eliminating a source of error that might have been introduced by the manifestation of the occasional stoicism found in the monkey.

The predominant response to each test applied is presented in Table 1 which demonstrates the changes that have occurred on the homolateral side subsequent to severance of the lateral corticospinal tract. The results

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† Medical Fellow of the National Research Council.

‡ Results of post-operative alterations in temperature regulation in these monkeys have been reported by Beaton and Leininger (1).

of certain of the more common tests are tabulated for each animal in Table 2, which illustrates the constancy or variability of the response.

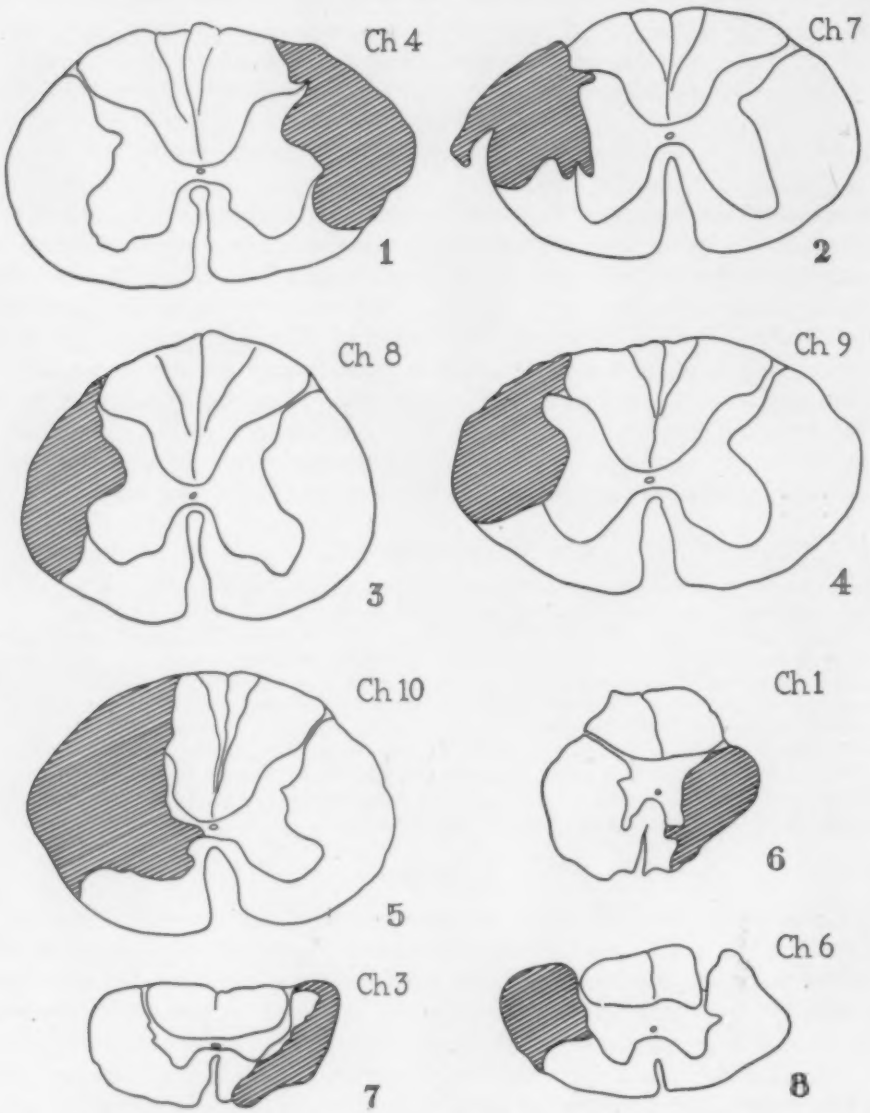

FIG. 1. Diagrammatic projection drawings showing destruction of the lateral cortico-spinal and adjacent ascending and descending tracts in the dorso-lateral column of the spinal cord.

In both cervical and thoracic animals the lower extremity of the operated side always exhibited a definite hypotonus on passive manipulation of all muscle groups. There was no evidence of clonus noted in either the

normal or affected limb. The usual response to tapping the patellar tendon on the side of operation was a slow to and fro swing of the lower leg in comparison with the quick jerk characteristic of the opposite or normal leg. In monkey Ch-4 this pendulous response was replaced by a mere diminution of the response obtained. The knee jerks of Ch-10 were of similar activity

Table 1

Test	Normal	Paretic
	Lower Extremities	
Passive manipulation	normal tone	hypotonus
Clonus	absent	absent
Pin prick	normal response	normal response
Patellar reflex	active	pendulous
Achilles reflex	active	hypoactive
Plantar stimulation	active	little response
Grasp	strong	absent
Placing reaction	present	absent
Hopping reaction	present	absent
Postural rigidity	absent	absent
Palpation of muscles	firm	flabby
Atrophy	absent	present
	Abdomen	
Abdominal reflex	active	absent
	Upper Extremities	
Passive manipulation	normal tone	slight hypotonus
Clonus	absent	absent
Pin prick	normal response	normal response
Biceps reflex	active	hypoactive
Triceps reflex	active	hypoactive
Palmar stimulation	normal	little response
Postural rigidity	absent	absent
Grasp	strong	diminished to absent

on both sides. The contraction of the adductors of the opposite thigh was noted to occur frequently in all monkeys when the patellar tendon of the affected limb was struck, but the reverse was not demonstrable.

Table 2

		Ch-1	Ch-3	Ch-4	Ch-6	Ch-7	Ch-8	Ch-9	Ch-10	Ch-11
Leg	Muscle tone	<nor.	<nor.	<nor.	<nor.	<nor.	<nor.	<nor.	<nor.	<nor.
	Clonus	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.
	Knee jerk	pend.	pend.	<nor.	pend.	pend.	pend.	pend.	nor.	pend.
	Grasp	abs.	abs.	<nor.	abs.	abs.	<nor.	abs.	abs.	abs.
	Plantar stimulation	<nor.	nor.	<nor.	nor.	abs.	<nor.	<nor.	nor.	<nor.
Arm	Muscle tone			<nor.		nor.	nor.	nor.	<nor.	
	Deep reflexes			nor.		<nor.	abs.	nor.	abs.	
	Grasp			<nor.		<nor.	<nor.	<nor.	abs.	

nor. = normal
<nor. = less than normal
abs. = absent
pend. = pendulous

The typical response to the placing of an object in contact with the plantar surface of the foot of the normal extremity of the monkey is a strong grasping of the object. The occasional atypical response to such a test is withdrawal of the foot in attempt to avoid the object. The hind limb suffering the paresis failed to show any response whatsoever in all animals except Ch-4 and Ch-8 in which a weak attempt to grasp was noted.

As has been reported by Hines (3), the usual response to plantar stimulation in the normal monkey is an initial extension and then a secondary flexion of the toes at the metatarsophalangeal joints. The affected foot of five monkeys of the present series gave a response much diminished in intensity and frequently not followed by the secondary flexion. Ch-7 gave no response. Ch-3, Ch-6, and Ch-10 showed a moderate response in both hind extremities.

The results of other tests applied, tabulated in Table 1, also deserve consideration. The Achilles tendon reflex of the paretic limb, when elicited in these animals, was hypoactive. The absence of the placing and hopping reactions in the affected limb with the presence of an active cross-placing response further demonstrates the loss of motor control. The abdominal reflex was elicited on the normal side in the animals tested but not on the paretic side. On palpation of the muscles of the calf and thigh, a flabbiness was noted in the limb of the operated side. Atrophy of these muscles was evident after about three weeks, especially noted in the triceps surae group.

The paresis occurring in the forelimb of the animals recovering from cervical tractotomy tended to be more obscure. Passive manipulation showed no indication of the loss of tone in monkeys Ch-7, Ch-8, and Ch-9. However, the paretic forelimb of Ch-4 and Ch-10 exhibited a decreased resistance in both the flexor and extensor group of muscles. The deep reflexes of these affected limbs varied in response from complete absence as in Ch-8 and Ch-10 to no change as in Ch-4 and Ch-9, with Ch-7 demonstrating the intermediate or hyporeflexia stage. Grasping with the paretic hand was very weak and inconsistent in all these animals and Ch-10 gave no response. Palmar stimulation resulted in little response.

In both the cervical and thoracic animals, tests for postural rigidity with the animal hanging in the hammock showed no increase in the tone of the paretic extremities. While hanging, the normal extremities were constantly being flexed. The paretic extremities, however, hung flaccidly extended and were never flexed.

Voluntary control of the movements of the arm at the shoulder suffered only a moderate loss. The loss was more evident at the elbow and was rather complete for digital movements. Gradual improvement was noted in the use of the arm, but digital paresis was permanent. The deficit of motor control was more apparent in the affected leg whose activity was limited to that of a strut in walking. No recovery or compensation was observed for the loss of digital control in the toes. The little tone that was noted some weeks after operation seemed to appear first in the abductors and flexors of

the thigh and flexors of the leg. This tone was never sufficient, however, to obscure the definite hypotonus that existed in comparison with the tone of the normal leg.

Discussion

It is evident from these observations that interruption of the lateral cortico-spinal tract in the spinal cord of the monkey gives rise to a paresis in the affected limbs. This paresis, which is more apparent in the lower than in the upper extremity, is characterized by a poverty of movement of the entire limb, and also by an apparent lack of voluntary control of movement. The paresis is less in the proximal muscle groups and more extreme in the distal, where it approaches a state of paralysis of all digital motion. There exists in these paretic limbs a diminished resistance to passive manipulation. This loss of tone is likewise indicated by the flabbiness of the muscles on palpation. In addition to this hypotonicity, the hypoactive or pendulous state of the tendon reflexes and the failure of clonus to develop, all definitely indicate the absence of spasticity after section of the lateral corticospinal tract.

The present results confirm those of Tower (4) who in 1940 reported that section of the pyramid in the monkey gave rise to a "hypotonic paresis" of the contralateral limbs. This term adequately describes the state found to exist in the present experiments after section of the cortico-spinal fibers within the dorso-lateral column at cervical or thoracic levels. It is generally believed that there are fibers from higher levels of the brain descending through each half of the spinal cord to the anterior horn cells and exerting an inhibitory influence upon their activity. Release from this inhibitory influence is said to result in a syndrome of spasticity characterized by muscular hypertonus, hyperactive reflexes, and clonus. If such fibers are present in the spinal cord of the monkey, the results of the experiments reported here indicate that they do not lie within the lateral cortico-spinal tract.

Summary

Interruption of the lateral cortico-spinal tract in the spinal cord of the monkey results in a paresis that is more prominent in the lower than in the upper extremity, and that is more pronounced in the distal than in the proximal muscle groups.

This paresis is characterized by hypotonicity, hypoactive reflexes, and absence of clonus, indicating that no descending inhibitory pathway whose interruption results in spasticity is present in the lateral cortico-spinal tract of the monkey.

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REMOVAL OF ACETYLCHOLINE BY CHOLINESTERASE INJECTIONS AND THE EFFECT THEREOF ON NERVE IMPULSE TRANSMISSION

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ALTHOUGH experimental results published during the past twenty years have presented strong indications that acetylcholine is produced in the course of the transmission of nerve impulses from postganglionic cholinergic nerves, from preganglionic endings in the autonomic nervous system, at voluntary motor nerve endings and possibly at central synaptic junctions, the controversy whether acetylcholine is essential to the process of transmission has not as yet been definitely settled.

We have attempted to re-investigate this question by utilizing highly purified cholinesterase preparations which would eliminate acetylcholine and thus afford a means of ascertaining its significance. If acetylcholine is essential for synaptic and postganglionic transmission in the parasympathetic nervous system, a reflex which is dependent on the integrity of this system should be abolished if acetylcholine were removed as quickly as it is produced. This, essentially, was the hypothesis tested in the experiments to be reported.

Before attempting to investigate the effect of the removal of acetylcholine on nerve impulse transmission, it was necessary to ascertain whether the cholinesterase preparations to be utilized as an instrument in this investigation could act on acetylcholine injected into the animal body. The present report is therefore divided into two parts: (i) the effect of cholinesterase injections on injected acetylcholine; (ii) the effect of acetylcholine removal on nerve impulse transmission. This latter effect was judged with specific relation to one reflex arc.

Cholinesterase preparations used

It has been demonstrated (3) that two types of cholinesterase exist in the animal body: (a) a specific enzyme which hydrolyses only certain esters of choline, exerting its maximum activity at acetylcholine concentrations as low as 3 mg. per cent, and displaying increasing inhibition at increased substrate concentrations; (b) a non-specific enzyme, pseudo-cholinesterase, which is capable of hydrolysing both choline and non-choline esters, exhibiting its optimum activity at acetylcholine concentrations above 300 mg. per cent—concentrations which are probably above physiological range. Both types of cholinesterase were used:*†

* The activity of the enzyme preparation was measured manometrically by Warburg's method at 37.5°C. in a 0.025 *M* $NaHCO_3$ solution saturated with 5 per cent carbon dioxide (pH 7.4). Each molecule of acid liberated from the acetylcholine during hydrolysis,

1. (a) a pseudo-cholinesterase from dog pancreas, prepared according to the method outlined by Mendel and Mundell (2); Q_{Ach} 500,000–700,000; (b) a pseudo-cholinesterase from horse serum, prepared according to a method as yet unpublished; Q_{Ach} 40,000–70,000; 2. a true cholinesterase prepared from the electric organ of *Electrophorus electricus*. A small amount of this material was made available to us through Dr. C. H. Best by Dr. D. Nachmansohn. The material was purified before using it in our experiments; $Q_{Ach} > 40{,}000$.

I. *Effect of cholinesterase injections on injected acetylcholine*

Acetylcholine when injected into rats brings about an excessive secretion of saliva, intensive lacrimation and the appearance of a material in the tears which greatly resembles blood. This material, an excretory product of the Harderian gland, has been identified spectroscopically as a mixture of protoporphyrin and coproporphyrin. Freud, according to Selye (4), described the shedding of so-called bloody tears after acetylcholine administration. Tashiro and his co-workers (5) proposed that the excretion, to which they applied the name "chromodacryorrhea" be used as a biological assay for acetylcholine. In uneserinized rats they detected the excretion after intraperitoneal injection of 2000γ acetylcholine or after intravenous injection of 10–15γ acetylcholine per 100 g. body weight. When administered intravenously to an eserinized rat, however, as little as 0.2γ acetylcholine per 100 g. body weight could produce this effect. In our experiments with uneserinized young rats, the reddish tinge characterizing this excretion could always be detected with filter paper within 5 minutes after the subcutaneous injection of as little as 250γ acetylcholine per 100 g. body weight.

In order to investigate the effect of injected cholinesterase preparations on the porphyrin excretion resulting from injected acetylcholine the following experiments were carried out: (i) a female rat, weighing 50 g. received 1 mg. acetylcholine chloride subcutaneously. Within $\frac{1}{2}$ minute, porphyrin excretion was evident, the pupils were constricted and excessive salivation was apparent. (ii) a female rat, weighing 50 g., received 1 ml. of an aqueous solution of 36.7 units of dog pancreas pseudo-cholinesterase intravenously. Two minutes later 1 mg. acetylcholine chloride was given subcutaneously. There was no abnormal salivation, no lacrimation nor evidence of porphyrin excretion; the pupils seemed larger and no light reac-

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releases one molecule of carbon dioxide from the bicarbonate. Therefore, the quotient,

$$\frac{\text{volume of } CO_2 \text{ (in } \mu l)}{[\text{time (in hrs.)}] \times [\text{dry wt. of enzyme preparation (in mg.)}]}$$

which will be denoted by the symbol Q_{Ach}, is a measure of the purity of the enzyme preparation.

The amount of enzyme capable of causing the evolution of 1 ml. CO_2 per minute at a substrate concentration of 1 per cent acetylcholine for the pseudo-cholinesterase and 10 mg. per cent acetylcholine for the true cholinesterase is defined as one unit of cholinesterase.

† Although the activity of the pseudo-cholinesterase is only slight at the low acetylcholine concentrations which probably occur under normal physiological conditions, we have compensated for its small activity at these levels by building up an excessive concentration of this enzyme in the experimental animals.

tion seemed evident. (iii) a female rat, weighing 50 g., received an intravenous injection of 1 ml. of an aqueous solution of the same dog pancreas pseudo-cholinesterase preparation, previously inactivated by heat. Two minutes later a subcutaneous injection of 1 mg. acetylcholine chloride was given. Within 5 minutes there was excessive salivation and porphyrin excretion, and the pupils were constricted.

These experiments which were repeated on different rats, with the same results, demonstrate that the porphyrin excretion normally following an

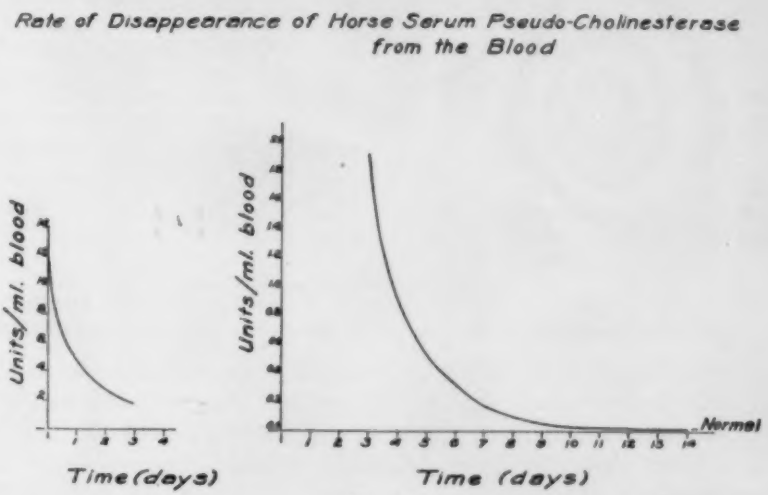


FIG. 1

injection of acetylcholine is prevented by an injection of pancreas pseudo-cholinesterase. It is evident that the absence of the porphyrin excretion is due to the enzyme action, since the inactivated preparation is unable to abolish this effect of acetylcholine. Changes in pupil size noted in these experiments were examined subsequently under different conditions and the results will be taken up later in this paper.

Porphyrin excretion could similarly be prevented by the injection of pseudo-cholinesterase from horse serum. Unlike the pseudo-cholinesterase from dog pancreas, which disappears almost completely from the circulation within one hour, the enzyme from horse serum leaves the blood stream very slowly (Fig. 1). The effects of a single esterase injection could therefore be followed at different blood-esterase levels in the same animal for a considerable length of time.

Four sets of experiments were carried out with different preparations of pseudo-cholinesterase from horse serum. Table 1 shows the data from one of these experiments. It may be seen that an injection of 60 units of horse serum pseudo-cholinesterase (Q_{Ach} 66,000) could protect the animal for 164 hours against the chromodacryorrhetic effect of acetylcholine.

Table 1

Time after injection of Ch-E	Cholinesterase, units/ml. blood at 1 per cent acetylcholine	Amt. of Ach. per 100 g. body wt. given subcutaneously	Reaction
27 hrs.	4.3	1 mg.	no salivation, lacrimination or porphyrin excretion
72 hrs.	1.92	1 mg.	slight salivation, but no porphyrin excretion
120 hrs.	0.46	1 mg.	salivation after ½ min., but no porphyrin excretion
144 hrs.	0.30	1 mg.	salivation after ½ min., lacrimation, but no porphyrin excretion
164 hrs.	0.17	1 mg.	salivation after ½ min., excessive porphyrin excretion
188 hrs.	0.09	1 mg.	salivation and porphyrin excretion before ½ min.
236 hrs.	0.03		
288 hrs.	0.02		
336 hrs.	0.008 (normal level)		

II. *Effect of removal of acetylcholine on nerve impulse transmission*

Since it was evident that the cholinesterase preparations were able to act on injected acetylcholine within the animal body, it seemed possible, through the injection of these preparations, to decide whether the removal of acetylcholine, physiologically produced, would have any effect on nerve impulse transmission.

Up to the present time the majority of the experiments designed to investigate synaptic transmission have been of an electrical nature. In our experiments, light, the natural stimulus for the reflex investigated was employed. The direct light reflex was brought into action and pupil sizes served as an indicator of the experimental results. Engelhart (1) demonstrated the presence of acetylcholine in the iris, ciliary body and aqueous humor of an eserinized rabbit's eye, which had previously been exposed to light but he could detect no acetylcholine in an eye which had not been subjected to illumination.

METHOD

Young albino rats, weighing 45–65 g., were used. The light source employed as the stimulus was maintained at a constant distance of 41 inches from the eye, the illumination being supplied by a 100W–120V bulb. The light intensity reaching the eye through the aperture in the screen was of the order of 2.5–3 foot candles, as measured with a photronic cell. An Argus model camera was mounted on an immovable base, to which an upright screen was attached at a constant distance from the lens. This screen was provided with a central opening through which the eye would show when the animal was placed directly behind it.

The results were recorded photographically on infra-red film, the pictures in both light and dark being taken with photoflash bulbs. The duration of the flash (1/50 sec.) allowed no time for constriction of the pupil since its outline proved to be sharp in photographs taken in the dark.

The experimental animals were not anaesthetized since pupil size deviates under

anaesthesia and variations in cell permeability under such conditions might also affect the action of injected cholinsterase. In unanaesthetized animals, emotional and psychical changes might give rise to variations in pupil size, but control runs on rats' pupils exposed to light showed that under the experimental conditions marked constriction was always obtained, though its extent varied from one animal to another.

The eye was exposed to the light source for 3 minutes, in order to obtain maximum constriction. A stronger stimulus could have achieved this effect in a shorter time but the animal might then have closed its eye or the unaccustomed light intensity might have served as a fright stimulus. In almost all experiments pupil sizes after the injection of inactivated preparations were recorded to prove that no material capable of causing dilatation was present in the solution. As a basis for comparing the results after injection of inactivated esterase with those obtained in situations involving similar psychical phenomena, pupil sizes after injection of Ringer's solution were recorded in some cases. All solutions used in these experiments were injected into a caudal vein.

Results

Figure 2 gives a diagrammatic representation of the pupil sizes obtained from a rat before and after an intravenous injection of 0.9 ml. of an aqueous

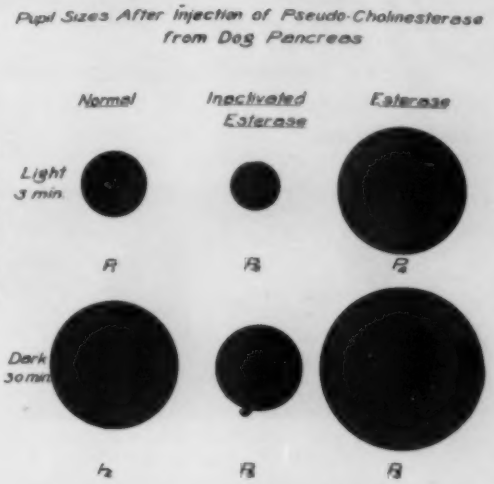


FIG. 2. P_4 and P_5 represent pupil sizes ten minutes after injection of the active and inactivated preparations respectively; P_5 and P_9 represent pupil sizes 45 minutes after injection of the active and inactivated preparations respectively. All diagrams in this figure are 17 times the original pupil size.

solution of 37 units of dog pancreas pseudo-cholinesterase and of 0.9 ml. of the same preparation, previously inactivated by heating for 30 min. at 60°C. It will be seen from this figure that after the injection of the cholinesterase the pupil size in the light (see P_4) approaches the pupil size recorded on the dark under normal conditions (see P_2). The pupil sizes after injection of the inactivated esterase seem to indicate the presence of some parasympatheticomimetic substances in the preparation. The experiments with active esterase, however, bear out that the enzyme is able to inhibit the normal light reflex in spite of the action of these constrictor substances. Figure 3 shows diagrammatically the comparative size of the pupil of a male rat before and after the injection of 33 units of horse serum pseudo-cholinesterase. The pupil sizes indicate that the pseudo-cholinesterase from horse serum is also capable of preventing the light reflex almost completely. The actual photographs taken in this experiment are shown in Fig. 4.

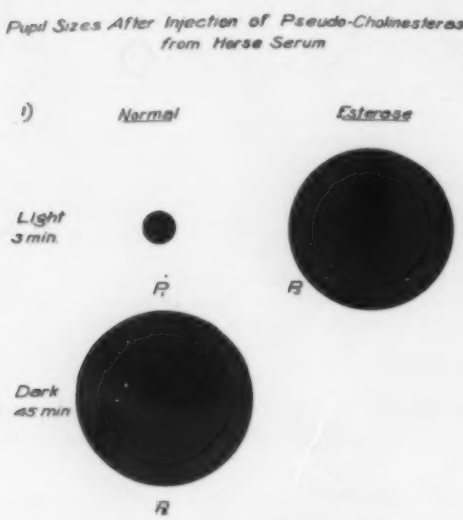
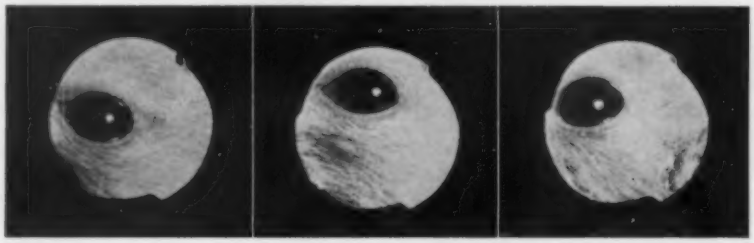


FIG. 3. P_2 was taken 35 minutes after the injection of the enzyme preparation. All diagrams in this figure are 17 times the original pupil sizes.

Figure 5 represents diagrammatically the pupil sizes of a rat before and after injection of 1 ml. of a solution of 60 units of horse serum pseudo-cholinesterase.



P_1 P_2 P_3

FIG. 4. Picture P_1 represents pupil size in the normal rat after an exposure to light of 3 minutes; picture P_2 represents pupil size in the same animal after an exposure to the dark of 45 minutes; picture P_3 represents the pupil size of the same rat after exposure of 3 minutes to light, following injection of 33 units of horse serum pseudo-cholinesterase.

Figure 6 shows the effect of 1 ml. of Ringer's solution and of 1 ml. of the esterase solution previously inactivated by heat, on the pupil size of another rat.

The latter figure demonstrates that the inactivated preparation causes no further effect on pupil size than the slight dilatation produced by the Ringer's solution. Any inhibition of the constrictor mechanism observed in Fig. 5 must therefore be due to the action of the active enzyme.

Figure 7 presents diagrammatically the pupil sizes found before and after the intravenous injection of 5.4 units of true cholinesterase obtained from the electric organ of *Electrophorus electricus*. Since only a limited quantity

of this material was available, it was not possible to investigate its action more fully, but it is evident from the experimental records that this enzyme is also capable of preventing the normal reaction of the pupil to light.

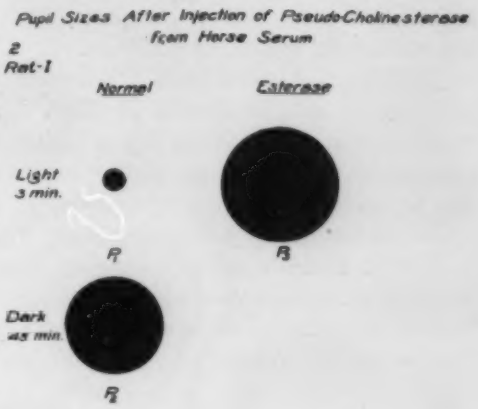


FIG. 5. P_3 represents pupil size in a rat 15 minutes after the injection of horse serum pseudo-cholinesterase. The diagrams in this figure are 20 times the actual pupil sizes.

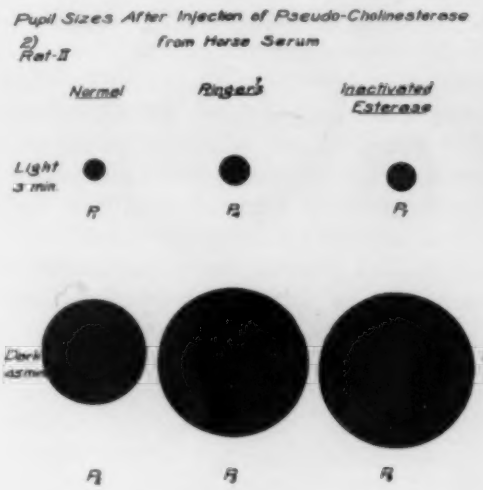


FIG. 6. P_4 and P_7 represent pupil sizes 28 minutes after the injection of Ringer's solution and inactivated esterase preparation respectively. P_3 and P_9 represent pupil sizes 15 minutes after injection of Ringer's solution and the inactivated enzyme preparation respectively, in the dark. The diagrams in this figure are 17 times the actual pupil sizes.

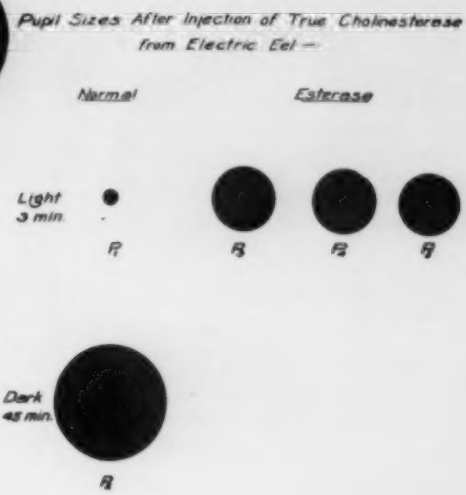


FIG. 7. P_3, P_4 and P_5 represent pupil sizes of the rat at 5 minute intervals, starting 3.5 min. after the injection of 5.4 units of true cholinesterase. The diagrams in this figure are 17 times the actual pupil sizes.

Discussion

Four points exist in the pathway of the direct light reflex where the transmission may be affected by the injected cholinesterase: (i) the synapses in the retina; (ii) the central synapses in the oculomotor nuclei; (iii) the synapses in the ciliary ganglion, and (iv) the endings of the short ciliary nerves on the sphincter pupillae.

Although it is impossible to decide from the experimental set-up at which point or points the esterase is effective, and although our experiments do not disclose the mechanism by which acetylcholine exerts its effect in the process of transmission, they do provide definite evidence that acetylcholine is essential for the transmission of the impulse to the sphincter pupillae, since its removal by cholinesterase abolishes the continuity of the impulse pathway. Similar experiments with cholinesterase injections on other effector organs innervated by cholinergic nerves must be undertaken, however, before any general statement regarding the role of acetylcholine in impulse transmission can be made.

Summary

Purified cholinesterase preparations, injected intravenously, are capable of acting within the animal body, thereby preventing the chromodacryorrhetic effect ordinarily obtained from injected acetylcholine.

It has been possible to prove, through the injection of these enzyme preparations, that acetylcholine plays an essential role in the transmission of nerve impulses to the sphincter pupillae. By measuring pupil diameters under constant experimental conditions, it has been demonstrated that the direct light reflex is partially or totally abolished by the injection of cholinesterase preparations, indicating that the integrity of the reflex depends on the presence of acetylcholine at some point or points in the pathway of the nerve impulse.

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THE BASIS FOR REPETITIVE ACTIVITY IN PHRENIC MOTONEURONS*

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TRAINS of impulses, conducted over phrenic and dorsal nerves to the diaphragm and intercostal muscles, produce rhythmic inspiratory expansions of the thorax. The frequency of impulses in any given motor unit and the number of motor units active determine the depth of inspiration (1, 3, 4). If the inspiratory center in the medulla oblongata is stimulated with repetitive shocks, these motor units respond; and the frequencies at which they respond, and the numbers which are active, are determined by stimulus frequency and intensity (10). These experiments suggest that a number of descending motor pathways connect the center with the several efferent neurons. They indicate also that the frequency at which any given efferent neuron is caused to fire is related to the average number of impulses reaching it per unit of time; *i.e.* to the number of these pathways activated, and to the frequency at which each transmits impulses. A limiting factor would logically be the instantaneous excitability of the efferent neuron at intervals after it has discharged an impulse. One might well assume a balance between these two factors as the final determinant of frequency.

Typically, phrenic neurons of the cat fire spontaneously at frequencies well below 50 per sec.; the majority of those which are active fire from 10 to 30 times per sec. at the peak of inspiration. If the above assumptions concerning the determination of discharge frequency are correct, one would predict that excitability is reduced, at least relatively, for periods as long as 100 m.sec. after the discharge of an impulse. With extreme asphyxial stimulation, however, discharge rates as high as 100 per sec. have been observed. Accordingly, one must assume that activation of a sufficient number of spinal respiratory pathways, at a high enough frequency, is capable of re-exciting phrenic neurons even during relative refractoriness. The present study is directed toward the quantitation of changes in excitability exhibited by phrenic motor neurons when they are subjected to volleys of impulses descending from the respiratory center. These experiments bear out the above predictions, and in addition throw further light on the nature of repetitive activity of motor neurons.

METHODS

Our experiments have been performed on cats anaesthetized with nembutal (30 mg. per kg.), administered intravenously. The 5th cervical root of the phrenic nerve was isolated, and the other roots sacrificed to obtain the greatest possible length of nerve in the

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neck. A condenser coupled amplifier and a commercial cathode ray oscilloscope were used for amplifying and recording potentials of the phrenic nerve. Brief condenser shocks (time constant, 0.1 m.sec.) were applied to the inspiratory center, or to the descending respiratory pathways in the spinal cord, through bipolar needle electrodes oriented in stereotaxic instruments. A two channel stimulator with independent output circuits was used in these experiments. The stimulator was triggered by a mercury switch activated by a respiratory tambour, and adjusted to deliver the stimuli in mid-inspiration or mid-expiration.

Results

Response of phrenic neurons to single shocks applied to the inspiratory center. A single shock applied to the inspiratory center leads to the discharge of a temporally dispersed volley of impulses over the phrenic nerve, lasting some 10 to 20 m.sec. As is evident from Fig. 1, the latency is brief during inspiration, and averaged about 3 m.sec. in this and in other experiments; while during expiration, it is prolonged to 6 to 9 m.sec. In this experiment, the conduction distance from the stimulating electrodes to the recording electrodes amounted to approximately 100 mm.; 55 mm. in the medulla and spinal cord, to the 5th cervical root; and 45 mm. over the phrenic nerve. Assuming a synaptic delay of about 1.0 msec. at the phrenic nucleus (7, 15), the latency during inspiration would suggest that the shortest effective pathway has probably one, and at most two, synaptic breaks. During expiration, on the other hand, the shortest effective pathway must include several neurons. An essentially maximal response was obtained during inspiration, with an arbitrary stimulus intensity of 15,* while during expiration, stimuli more than three times this intensity produced lesser responses. Thus certain phrenic motor neurons are relatively inaccessible to single volleys of impulses from the inspiratory center in the absence of some facilitative background of respiratory activity.

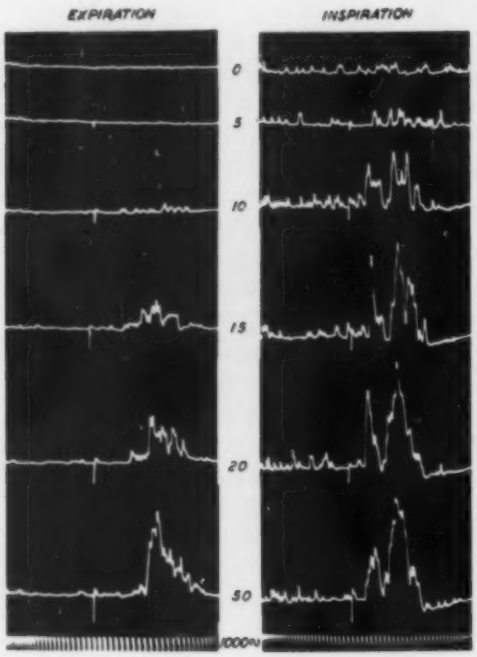

Fig. 1. Phrenic nerve response to single shocks of various intensities applied to the inspiratory center during the mid-expiratory and mid-inspiratory phases of the respiratory cycle. Intensities in arbitrary potentiometric units; 50 potentiometric units, approximately 10 peak V.

Facilitation of phrenic neurons. We have studied the time course and degree of facilitation of phrenic neurons by quantitating the nerve response to

* In this experiment 5 potentiometric units correspond approximately to a peak load voltage of 1V.

a constant testing stimulus, introduced into the inspiratory center at various intervals after a constant conditioning stimulus. Obviously, the height of a response, as temporally dispersed as those shown in Fig. 1, is not a very good indication of number of responding neurons, and hence of the average excitability of the segmental population. Area would seem to be a more quantitative measure of neuron activity. Accordingly response areas were measured, by means of a planimeter, on enlarged tracings of the records. Measurement was complicated by the necessity for introducing a variable factor to correct for non-linearity of the sweep. Since the response itself is inherently somewhat variable, routinely three records were taken at each shock interval, and the response areas for the three averaged.

Figure 2 (C to K) shows the response to a threshold testing shock applied

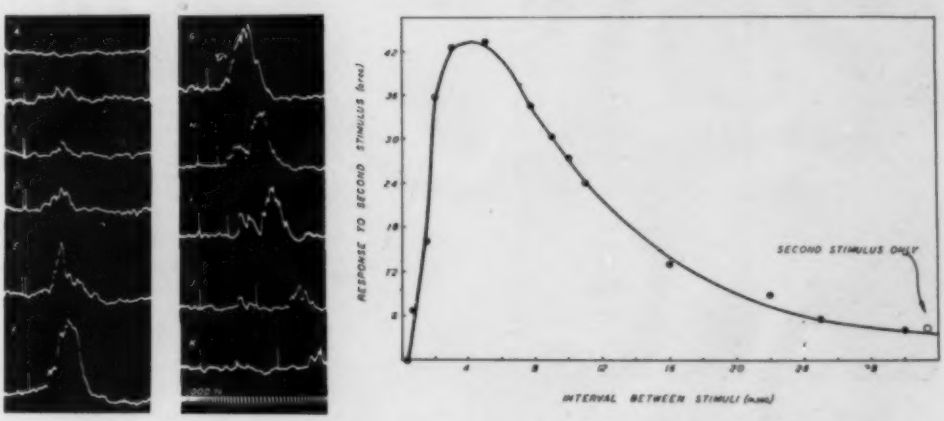

FIG. 2. Phrenic nerve response to two equal shocks of threshold intensity applied to the inspiratory center in succession at intervals of 0.5 to 25 m.sec. Shocks applied during mid-expiration. In the graph the area of the response to the second or testing shock is plotted as a function of the interval between it and the first or conditioning shock.

to the medulla at varying intervals after a conditioning shock of equal threshold intensity. All records were taken during mid-expiration. The average total response area, minus that of the response to the conditioning shock alone, *i.e.*, the area of the conditioned test response, is plotted in the graph on the right, against the interval between the two shocks. Areas are expressed in arbitrary units which are comparable only within a given series of experiments. Facilitation is first evident when the conditioning shock precedes the testing shock by 1.5 m.sec. (E), increases to a maximum when the interval is from 3 to 5 m.sec. (F and G), and disappears after 25 to 30 m.sec. While facilitation may be readily demonstrated during both inspiration and expiration, it is most apparent in the latter phase, when the inspiratory center is quiescent.

The greater magnitude of phrenic nerve response to a single shock during inspiration than during expiration (Fig. 1), finds explanation in the facilita-

tive action on phrenic neurons of the normal inspiratory center discharge. Furthermore, the discharge of these motoneurons, in response to a single shock, is limited to the interval during which facilitation from that shock exists. It would seem that the neurons responding, after all but the briefest latencies, do so only after some degree of facilitation has been developed. This relatively long lasting facilitation finds explanation in terms of prolonged delivery of interneuron impulses to the motoneurons over delay pathways or by way of reverberating circuits (8, 14).

Subnormality in phrenic neurons. If the conditioning shock is increased in intensity, and delivered during inspiration, so as to cause a fair proportion

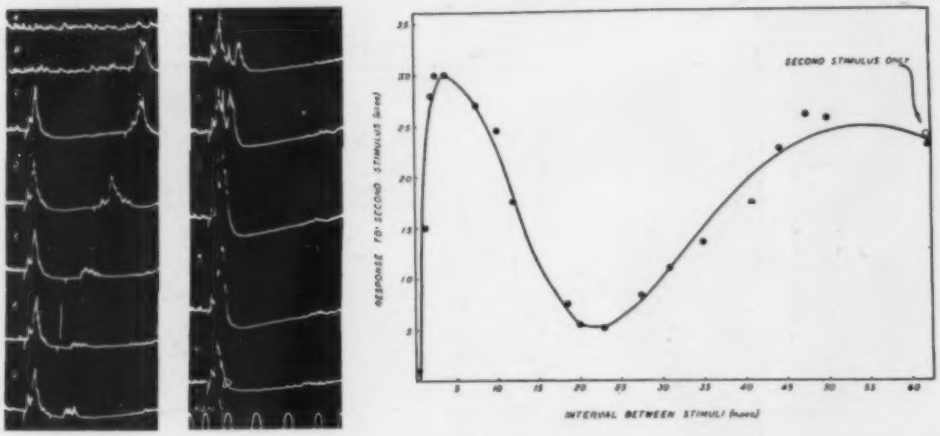

FIG. 3. Phrenic nerve response to a testing shock of moderate intensity (3V) following a stronger conditioning shock (8V) at various intervals. Shocks applied during mid-inspiration. In the graph the area of the response to the testing shock is plotted as a function of the interval between the shocks.

of the phrenic neurons to respond; and if the testing shock is also increased somewhat in intensity, a different cycle of excitability change occurs. Figure 3 (C to L) illustrates such an experiment, plotted, on the right, in a fashion identical to that of Fig. 2. The test response, unconditioned in record B, is progressively reduced (C to F), as the interval between conditioning and testing shocks is reduced. This extinction of response is most marked when the interval is 20 to 25 m.sec., as shown on the graph. When the interval is further reduced, the testing response increases in magnitude (G to K), and may show definite facilitation when the interval is about 5 m.sec. With still shorter intervals, the response drops off again. One is tempted to assume by comparing Fig. 2 and 3, that two processes, altering excitability in opposite directions, are simultaneously initiated by strong conditioning stimuli. A weak conditioning stimulus, causing few phrenic neurons to respond, accentuates the facilitative process, Fig. 2. A strong conditioning stimulus, causing many phrenic neurons to respond, brings into play simul-

taneously both facilitation and subnormality. Early in recovery from such a strong conditioning stimulus, a more short-lived facilitation might outweigh depression of excitability; but later, a longer lasting depression would become more evident. It is interesting to observe that the spontaneous discharge of impulses into the phrenic nerve (Fig. 3A) is abolished for the duration of the period of subnormality (note especially H to L).

Facilitation and subnormality in single motor units. To relate these average

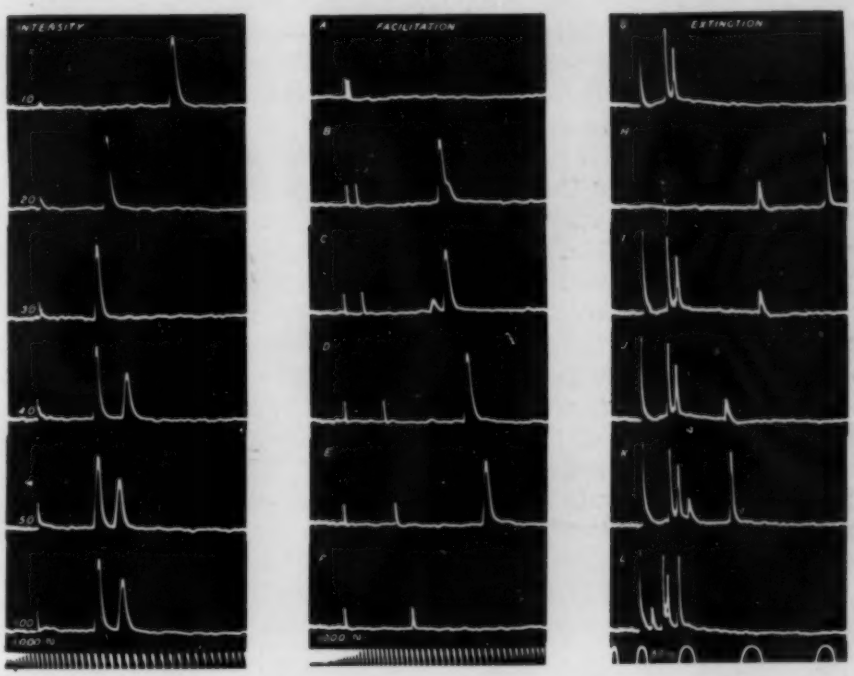

FIG. 4. Response of two phrenic neurons to shocks applied to the inspiratory center during mid-expiration. *Intensity* records, single shocks at various intensities from 10 to 100 arbitrary potentiometric units (approximately 3 to 30V). *Facilitation* records, two equal subthreshold shocks at intervals of 1 to 16 m.sec. *Extinction* records, conditioning shock of 15V followed at various intervals by a testing shock of 6V. The first deflections in records *G* and *H* are the shock artifacts of the conditioning and testing shocks respectively.

changes in excitability of a segmental population of motoneurons to the behavior of the individual units of the group, we have studied some eight single or double phrenic neuron preparations under similar experimental conditions. In Fig. 4 are presented records from one such preparation, in which the responses of two neurons, distinguishable by differences in spike potential, are evident. Gradual deterioration of the end of the fiber accounts for the reduced response of the neuron of lesser potential in the records titled facilitation. All records were taken during mid-expiration. In the first series of records, single shocks of progressively increasing intensity were applied to the inspiratory center. Intensities, in arbitrary potentiometric units, are

given at the left. An intensity of 10* constituted threshold for the neuron of greater spike potential; 40, for the one of lesser spike potential. Latency diminished from 18 m.sec. at threshold, to 9 m.sec. at an intensity of 30, for the neuron of larger potential. For the other neuron latency shortened only slightly at intensities above threshold. The development of a critical degree of facilitation can evidently be hastened considerably in certain instances as a result of increasing the number of descending respiratory pathways active (*i.e.* by increasing stimulus intensity). In no preparation have we observed a repetitive response from a single shock, no matter how high the intensity. Thus the temporally dispersed character of the discharge, shown in Fig. 1, represents for the most part, variation in the time necessary for the several neurons to attain this critical state of facilitation.

In the second group of records titled *facilitation*, two stimuli of approximately half of minimum threshold intensity (one eighth threshold for the neuron of lesser spike potential), were introduced into the respiratory center at varying intervals. When the intervals between conditioning and testing stimuli amounted to 3 to 14 m.sec., the neuron of greater spike potential responded (B to E). Within the restricted limits of 3 to 6 m.sec., the other neuron, of much higher threshold and lesser spike potential, also responded. The response of this neuron may be observed as a small deflection on the down slope of the spike in record B, which precedes the spike in C.

In the third group of records titled *extinction*, a strong conditioning stimulus, to which both neurons responded (record G), was followed at varying intervals by a moderate test stimulus, to which only the neuron of lower threshold responded (record H). With intervals from 80 to 20 m.sec. the response to the testing shock was extinguished; while with intervals from 20 to 3 m.sec. a testing response was again obtained. When the testing stimulus followed the conditioning stimulus at intervals less than 3 m.sec., no response was obtained.

Similar experiments during the mid-inspiratory phase of the respiratory cycle were difficult to interpret, for it was impossible, with apparatus available, to introduce a stimulus into the inspiratory center, in fixed relation to a spontaneously fired phrenic neuron impulse. Hence no control could be exerted over the spontaneous fluctuations in excitability of the neuron in question. Results qualitatively similar to those of Fig. 4 were obtained in the other seven experiments.

However, as one might anticipate, the intensity of the testing stimulus largely determined the apparent duration of subnormality; and if a testing stimulus of sufficiently high intensity were employed, no phase of extinction of response could be demonstrated in some neurons, beyond a short initial period of unresponsiveness. The use of the single neuron preparation is obviously of limited application in determining the varying magnitude or the time course of an excitability change, since its response is of an all or none

* In this experiment 10 potentiometric units correspond approximately to a peak load voltage of 3V.

character. Accordingly experiments were performed, utilizing the entire 5th root of the phrenic nerve, to determine in what way intensity of testing and conditioning stimuli modify these apparent changes in excitability.

Influence of testing shock and conditioning shock intensity on apparent excitability change. Figure 5A is a comparison of the apparent changes in excitability, following a near threshold conditioning stimulus, when they are measured by testing shocks near threshold (open circles), and when they are measured by shocks of moderate intensity* (solid circles). The stimuli were applied to the inspiratory center during mid-expiration. For both curves,

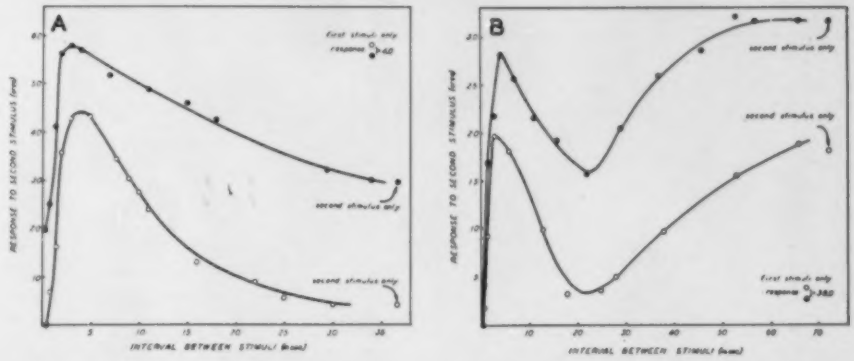

FIG. 5*A*. A comparison of the changes in excitability resulting from threshold conditioning shocks when measured by testing shocks near threshold (open circles) and of moderate intensity (solid circles). Stimuli were applied to the inspiratory center during mid-expiration.

FIG. 5*B*. A comparison of the changes in excitability resulting from strong conditioning shocks when measured by testing shocks of moderate intensity (open circles) and high intensity (solid circles). Stimuli were applied to the inspiratory center during mid-inspiration.

the response to the conditioning stimulus in isolation amounted in arbitrary area units to 4. The response to the weaker testing stimulus in isolation was also 4, while to the stronger testing stimulus the response amounted to 30. It is apparent from these curves that the average time course of the facilitation which results from such low intensity conditioning stimuli is not altered by the choice of the intensity of the testing shock, though the relative degree of facilitation is greater when measured with weaker testing shocks. Similar conclusions may be drawn concerning the effect of testing shock intensity on the measurement of excitability after strong conditioning stimuli, applied to the inspiratory center during mid-inspiration. Figure 5B

* In this and succeeding experiments we have expressed intensities in relative descriptive terms. We have done so because we feel that peak load voltages have little significance in the absence of knowledge of exact distances of the stimulating electrode tips from the excitable structures. Slight variations in the position of the electrodes greatly alter threshold stimulus intensity (9), and obviously in our several experiments these variations have been significant. Intensities in all our experiments have been within the range of 1 to 30V, for the most part within 1 to 15V.

compares the excitability changes following a strong conditioning stimulus, when measured by testing shocks of moderate intensity (open circles), and when measured by shocks of high intensity (solid circles). The times of the initial peak, the succeeding depression, and the final return to normal excitability are not significantly different in the two instances, although the changes are accentuated by using weaker testing stimuli.

Choice of intensity of the testing shock alters apparent magnitude but not time course of the excitability changes which follow conditioning shocks of fixed intensity; choice of intensity of the conditioning shock obviously alters both magnitude and time course, and as well, the character of these

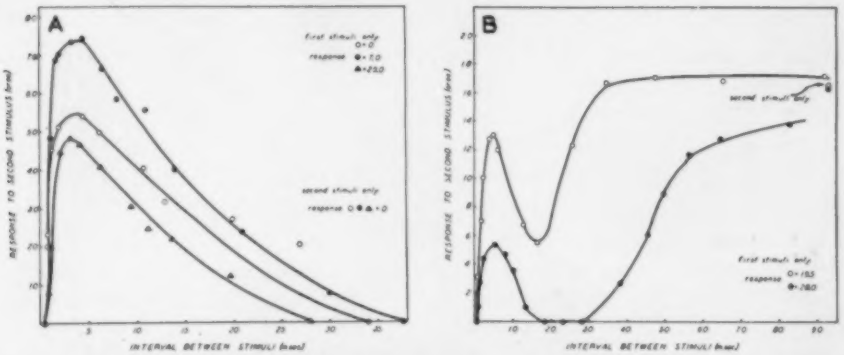

FIG. 6*A*. A comparison of the changes in excitability resulting from conditioning shocks of three intensities: subthreshold (open circles); low intensity (solid circles); moderate intensity (triangles). The testing shocks were equal and subthreshold for all. Stimuli were applied to the inspiratory center during mid-expiration. Note that the degree of facilitation at first was increased and then diminished as a result of progressive increase in intensity of the conditioning stimulus.

FIG. 6*B*. A comparison of the changes in excitability resulting from strong conditioning shocks (open circles) and essentially maximal conditioning shocks (solid circles). The testing shocks were moderate and of such intensity as to permit quantitation of both increases and decreases in excitability. Stimuli were applied to the inspiratory center during mid-inspiration.

changes. In the experiment shown in Fig. 6A, just subthreshold shocks were used to test excitability following conditioning shocks of three intensities: just subthreshold (open circles), low intensity (solid circles), and moderate intensity (triangles). All stimuli were applied to the inspiratory center during mid-expiration. Marked facilitation follows conditioning shocks of all three intensities, but there is evident a somewhat perplexing variation with intensity in the degree of facilitation. Facilitation increased as the intensity of the conditioning stimulus was raised from below threshold values to slightly above threshold; and then decreased as intensity of the conditioning stimulus was further raised, as may be seen in the lower curve of Fig. 6A. This apparent decrease in facilitation sets in at an intensity of the conditioning stimulus which leads to significant motoneuron discharge; and in general, the reduction is related to the magnitude of that discharge.

The process may be followed further by increasing the intensity of the test stimulus to such a value that both increases and decreases in excitability may be quantitated. Such an experiment is illustrated in Fig. 6B, in which, during mid-inspiration, the excitability changes were followed after strong (open circles), and essentially maximal (solid circles) conditioning shocks. Both degree and duration of subnormality increased when greater numbers of motoneurons were caused to fire by increasing the intensity of the conditioning shock.

In the experiments outlined above we have arbitrarily chosen to demonstrate increases in excitability following weak conditioning shocks by delivering them to the inspiratory center during mid-exspiration. To demonstrate depression of excitability, on the other hand, strong conditioning

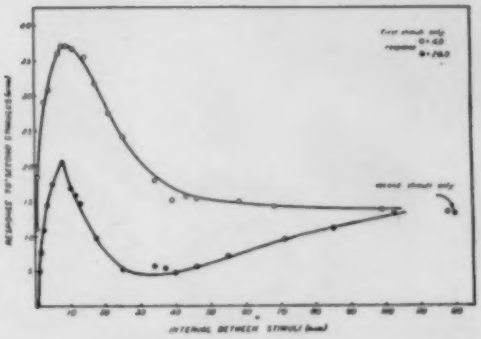

FIG. 7. A comparison of changes in excitability produced by threshold and by strong conditioning shocks. The testing shocks were moderate and of such intensity as to permit quantitation of both increases and decreases in excitability. Stimuli were applied to the inspiratory center during mid-expiration.

shocks have been applied during mid-inspiration. The reason for this choice lies in the fact that even weak conditioning stimuli, introduced during inspiration, cause the firing of appreciable numbers of phrenic motoneurons. These neurons are apparently already somewhat facilitated by the impulses from an active inspiratory center. Hence even though an initial phase of facilitation may be demonstrated following the weak stimulus, it is succeeded by appreciable subnormality. On the other hand, a weak or even moderate stimulus during expiration may produce marked facilitation; yet, since few motoneurons discharge, no reduced excitability follows. By proper choice of intensities of both conditioning and testing stimuli, however, the same phenomena may be demonstrated in either phase of the respiratory cycle.

Dual nature of excitability change following strong shocks. To illustrate further the dual nature of the changes in excitability produced by strong stimuli, two intensities of conditioning stimuli were chosen, one of which produced facilitation alone, the other, facilitation followed by subnormality. The stimuli were applied to the inspiratory center during mid-expiration, and excitability was quantitated by a constant test stimulus. The experimental data are shown in Fig. 7, and the smoothed curves are plotted as A and B, in Fig. 8. In the latter figure, the magnitude of the unconditioned test response

is plotted as the zero reference level. Variations above this reference level indicate facilitation; variations below, indicate subnormality. Subtracting curve B from A, yields C. The actual form of the initial part of curve C, and the extent of the subnormality which it indicates should be considered as having only qualitative significance. For instance, slight displacements of curves A and B which are well within the range of experimental variability, may considerably alter the initial part of curve C. Also it is quite reasonable

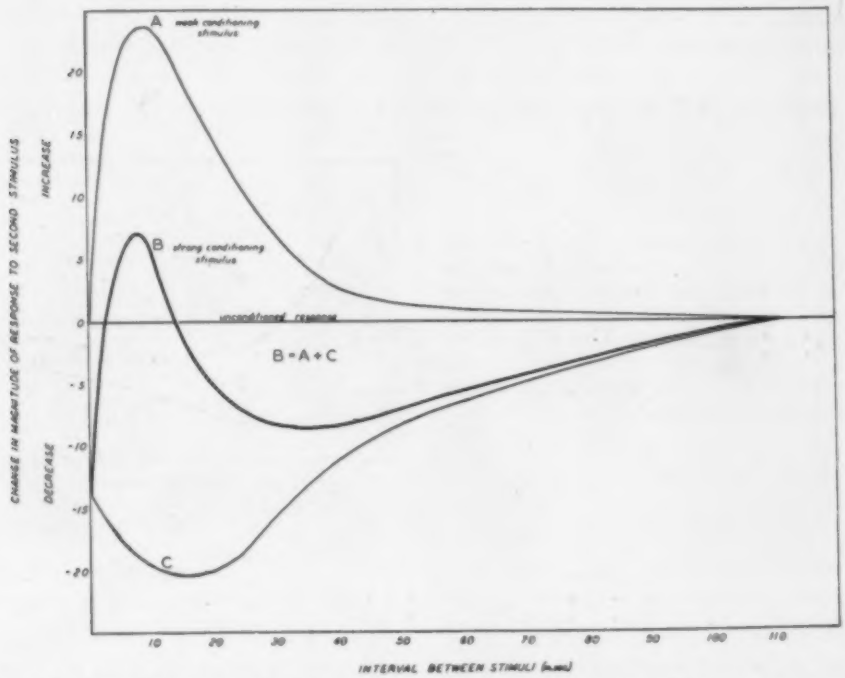

FIG. 8. A graphic analysis of the changes in excitability produced by weak and strong conditioning shocks applied to the inspiratory center. Data obtained from Fig. 7; explanation of the analysis in the text.

to assume that the actual magnitude of the facilitative process is greater with the stronger conditioning shock than with the weaker. Hence the extent of the depression of excitability is really much greater than that shown by curve C. But such a plot does illustrate that a strong conditioning stimulus initiates two independent processes; one, characterized by increased excitability, the other by depressed excitability. In all of our experiments, the latter change seems correlated with the numbers of responding motoneurons.

Site of excitability change. The possible sites at which these changes in excitability might occur, and hence the structures involved are sufficiently few in number to encourage an attempt at their elucidation. Experiments to be presented indicate that facilitation results largely from repetitive activity of inspiratory center neurons, and only to a minor degree from repetitive

spinal interneuron activity. Subnormality, on the other hand, is largely resident in phrenic motoneurons, and to a lesser extent in spinal interneurons and respiratory center neurons. A number of experiments were performed in which the spinal cord was systematically stimulated at the first cervical level with minute needle electrodes oriented to various depths with an adjustable carriage attached to the Horsley-Clarke instrument. A response latency, significantly shorter than that observed on stimulation of the inspiratory center, was the criterion chosen to indicate a descending respiratory tract response.

In initial experiments, exploration of the cord was carried out with single

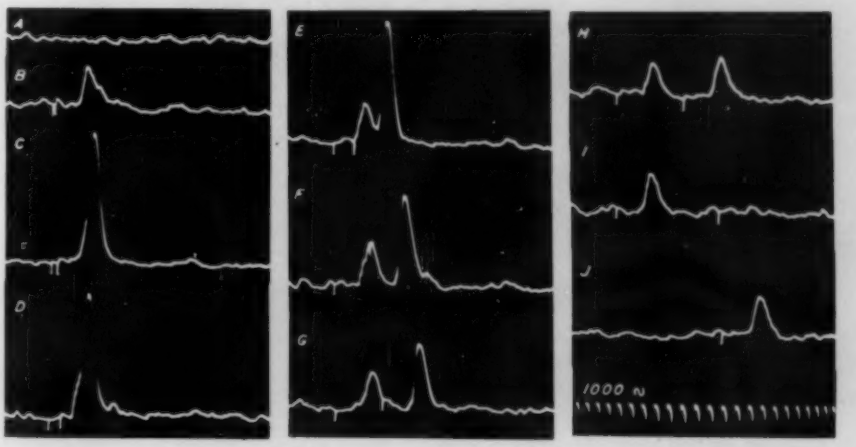

FIG. 9. Phrenic nerve response to two equal shocks of low intensity applied in succession to the descending spinal respiratory pathways at intervals of 0.3 to 8.0 m.sec. Stimuli applied during mid-inspiration.

shocks applied during mid-expiration. Under these conditions, no responses of short latency were obtained from any part of the cord. However, from the lateral columns there were obtained responses similar to those shown in Fig. 1, but with significantly longer latencies. These responses undoubtedly represent effects of stimulating afferent pathways with relay through the respiratory center. In subsequent experiments, in which shocks were applied during mid-inspiration, responses of brief latency were obtained from the anterior and antero-lateral columns of the cord. These responses differed from those obtained on stimulation of the inspiratory center in that the latency was shorter and the discharge more nearly synchronous. In Fig. 9 are presented records obtained when two equal shocks of low intensity were applied to the anterior columns during mid-inspiration. The response to the conditioning shock alone is shown in record I; to the testing shock alone, in record J. With intervals of 0.3 m.sec. or less, no response was obtained to the testing shock. When the interval amounted to 0.7 to 1.7 m.sec. definite facilitation was observed (C, D, E), which diminished as the interval was prolonged to

5 m.sec. (F, G, H). Extinction of the testing response occurred at 8 m.sec and for a considerable period thereafter. Since latency amounted to about 2 m.sec. in this experiment for a total conduction distance of 70 mm., the response to a single volley of impulses (I, J) must represent direct activation of some phrenic neurons by endings of tract fibers. The facilitated responses (C to G) probably represent the addition of other neurons discharged as a

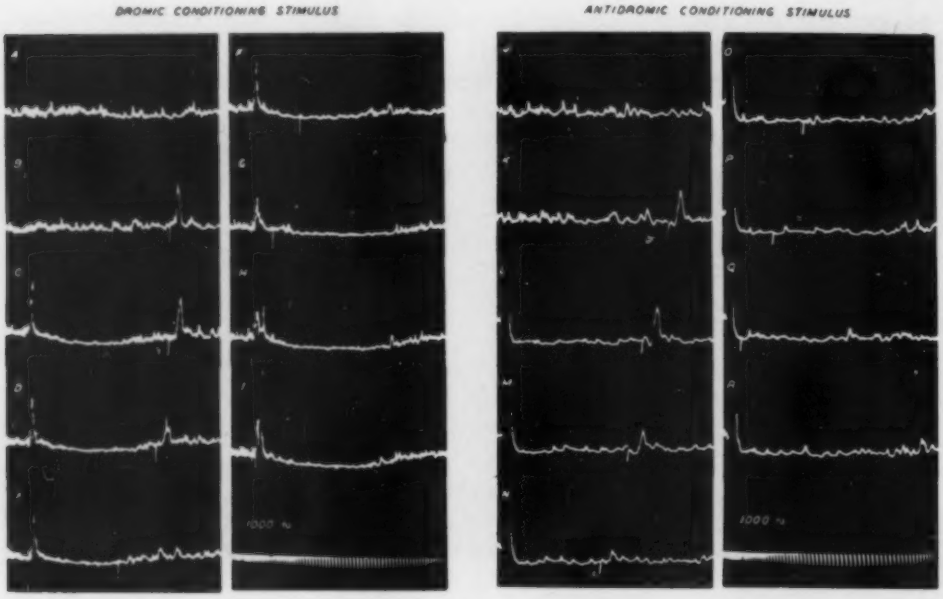

FIG. 10. A comparison of degree and time course of subnormality of phrenic neurons induced by dromic and antidromic excitation. The testing shocks in both instances were applied to the spinal respiratory pathways. In the records of dromic excitation, the conditioning shocks were applied to the spinal pathways through the same electrodes. In the records of antidromic excitation, the conditioning shocks were applied to the phrenic nerve.

result of coincidence of the second tract volley and local internuncial impulses set up by the first volley. For the very brief shock intervals in records C and D, a single relay interneuron is probably involved. For the intervals shown in records E to G, a short chain of interneurons probably continues to deliver impulses for some 6 m.sec.

A comparison of the records of Fig. 2 and 9, however, illustrates the fact that facilitation (as indicated by an increase in response area) is less striking and much less persistent following a conditioning shock to the descending respiratory tracts, than when applied to the center. This is emphasized by the fact that significant responses could not be obtained with single stimuli applied to the tracts during expiration, *i.e.* in the absence of a facilitative background of inspiratory activity. We may assign the major part of the facilitation produced by a single conditioning stimulus in the medulla to

repetitive activity of inspiratory center neurons. These neurons have been shown to be extensively interconnected (10, 12) and are probably arranged in reverberating or reentry circuits.

Subnormality in these experiments did not seem to be essentially different from that observed in previous experiments (*cf.* Fig. 3), except for its earlier appearance (record 9I). To determine whether this subnormality following discharge of phrenic motoneurons is resident in these neurons or in the surrounding internuncial pool, excitability was tested by a tract shock, following antidromic and tract conditioning shocks. The 4th, 5th and 6th cervical dorsal roots were sectioned ipsilaterally, and the 5th cervical phrenic root used for recording and stimulating. One such experiment is illustrated in Fig. 10. On the left (records A to I), a strong tract conditioning shock was followed by a moderate tract testing shock. On the right (records J to R), the same testing shock was applied following an antidromic conditioning shock applied to the phrenic nerve. Due to blocking of the amplifier, a maximal antidromic shock could not be used. All records were made during mid-inspiration.

As may be seen by comparing the two sets of records, essentially the same time course of reduced excitability was observed following transsynaptic and antidromic excitation of phrenic neurons. Despite the difficulties in interpreting results of antidromic excitation (6), these experiments would seem to point to the phrenic neurons as the site of the major depression of excitability. However, these experiments do not rule out the possibility that respiratory center neurons or local spinal interneurons may also show subnormality as a result of intense activity initiated by a strong conditioning shock.

Discussion

The repetitive initiation of nerve impulses by a sensory receptor has been explained by Adrian and Zotterman (2) in terms of the time course of recovery of excitability of the receptor and the intensity of the maintained stimulus. For a brief interval after an impulse has been initiated, the threshold of the receptor is infinite; recovery then begins, and the threshold falls gradually toward the resting level. The higher the intensity of the maintained stimulus, the sooner within the recovery period will it exceed the decreasing threshold of the receptor, and the earlier will another impulse be initiated. As was inferred by these investigators, this concept may be applied to repetitive discharge of motor neurons, if one substitutes for the constant stimulus acting on the sensory receptor, a more or less statistically constant barrage of impulses impinging on the motor neuron by way of the many end feet covering its soma and terminal dendrites. The frequency at which impulses are initiated by the motor neuron becomes a function of the time course of recovery of its excitability and the average intensity of this synpatic bombardment. This concept has been applied in explaining the discharge of phrenic motoneurons resulting from stimulation of the in-

spiratory center with repetitive stimuli of various intensities and frequencies (10).

The present investigation throws light on the origin of the synaptic barrage impinging on the final motoneuron, and on the magnitude and time course of subnormality of that neuron once it has been caused to fire. A diagrammatic representation of these relationships is given in Fig. 11 and 12. A strong shock applied to the center *A* in Fig. 11 leads to a protracted volley of impulses, which reaches its greatest magnitude at about 5 m.sec., and must continue with diminishing intensity for 30 or more m.sec. Some direct tract fibers impinge on motoneuron *B* and interneuron *C*, but in the absence of spontaneous activity in the system, the impulses delivered by the direct collaterals serve only to lower the threshold of the motoneuron to succeeding impulses from center and interneuron. Thus latency is longer in expiration than in inspiration (Fig. 1); for in the latter phase, an already facilitated motoneuron responds to the initial direct tract impulses. Spinal interneurons must be relatively few in number for facilitation is brief following a single shock to the cord, lasting only a few m.sec. (Fig. 9).

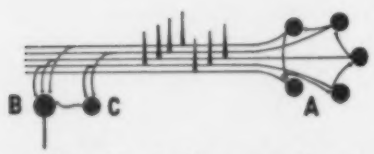

FIG. 11. A diagrammatic representation of inspiratory center phrenic neuron relationships. *A*, inspiratory center, the neurons of which are connected in a reverberating circuit such that a single shock applied to the center leads to a protracted volley of impulses over the descending spinal pathways. *B*, phrenic neurons, receiving some direct respirator[illegible]ract collaterals and also terminations of local spinal interneurons, *C*.

An exact measurement of the absolute refractory phase for phrenic

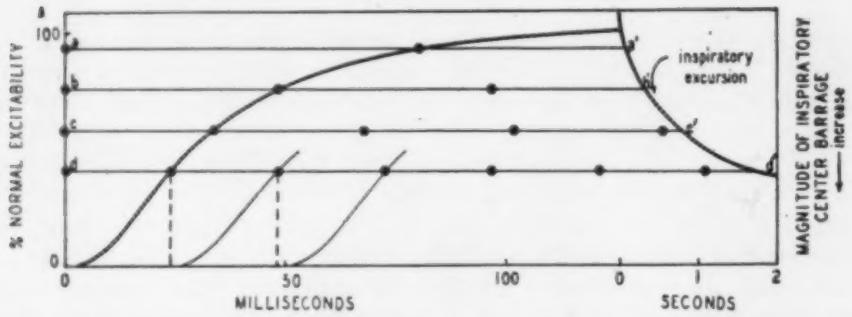

FIG. 12. A schematic representation of those factors which play a role in the regulation of frequency of phrenic motoneuron discharge (modified after Adrian and Zotterman, 2). The heavy curve represents recovery of excitability of a phrenic neuron once it has discharged an impulse. The horizontal lines represent the magnitudes of the inspiratory center barrage impinging on that neuron at various times during the developing inspiration. The neuron will again fire an impulse when it recovers to a point where its instantaneous excitability is equal to the maintained level of excitation. The dots on the horizontal lines represent the times at which impulses will be discharged by the neuron at successive intervals during inspiration.

motoneurons has not been possible but it would seem to lie between 1 and 3 m.sec. But relative refractoriness, diminishing in magnitude, lasts up to 100 m.sec. following discharge (Fig. 7). For about 1 m.sec. following a strong shock to the center, no response to a second shock may be obtained. However there continues to be delivered to the motoneuron an increasing barrage of impulses, which more than offsets the depressed excitability after 5 m.sec. or so. As this barrage diminishes in magnitude, the more prolonged depression of excitability becomes evident, and may completely extinguish the response to the testing shock at about 30 m.sec. (Fig. 8). This depression of excitability is presumed to be resident chiefly in the phrenic motoneurons on the basis of antidromic excitation experiments.

The conditions which obtain in normal respiration are obviously considably different from those described above. A diagrammatic representation of the factors controlling normal phrenic motoneuron discharge are given in Fig. 12. During inspiration, the discharge of impulses from the center progressively increases, paralleling the degree of inspiratory expansion of the thorax ($a'd'$), until the inhibitory inflow from pulmonary afferents and the brainstem inhibitory system (pneumotaxic center) cuts them off sharply, d' (11, 13). The horizontal lines a–a', b–b', c–c', d–d' represent magnitudes of this barrage of impulses (*i.e.* total number of impulses per unit time) impinging on a given phrenic neuron at any instant. These levels of excitation may be considered in relation to the curve on the left which represents recovery of excitability of that neuron once it has discharged an impulse. The neuron will again fire an impulse when it recovers to a point where its instantaneous excitability is equal to the maintained level of excitation. Thus the dots on the horizontal lines represent the times at which succeeding impulses will be discharged for each level of excitation during the developing inspiration. Such a plot illustrates the basis for the characteristic slowly augmenting and rapidly decreasing frequency of discharge of phrenic units during the inspiratory cycle (5). The factors underlying the phasic variation in excitatory outflow from the inspiratory center have been treated elsewhere (11, 13).

Summary

A single shock, applied to the inspiratory center in the medulla oblongata of the cat, leads to the discharge of impulses over spinal respiratory pathways for periods of 30 m.sec. or more. If the stimulus is weak and applied during expiration, it will cause few phrenic neurons to respond, but will facilitate those neurons to subsequent shocks if they follow the first at intervals of less than 30 m.sec. On the other hand, a strong stimulus produces this same facilitation, but since it causes large numbers of phrenic neurons to respond, it initiates subnormality in those neurons. For an initial period of 20 m.sec., the more short-lived facilitation outweighs subnormality, but the latter dominates the picture during the succeeding 100 m.sec.

Facilitation largely results from the continued delivery of impulses from

center to motoneuron as a result of delay pathways or reentry circuits within the center. Spinal interneuron repetitive activity plays a much less prominent role. Subnormality on the other hand is mainly resident within the phrenic motoneurons.

The repetitive discharge of phrenic neurons which characterizes normal inspiratory activity may be explained in terms of a balance between the degree of excitation of those neurons and their rates of recovery of excitability.

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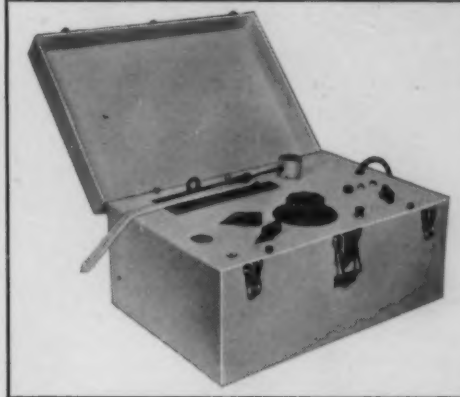

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